AUG 8 1944

CANCER RESEARCH

Medical Library

VOLUME 4
NUMBER 8
AUGUST, 1944

A MONTHLY JOURNAL
OF ARTICLES AND ABSTRACTS
REPORTING CANCER RESEARCH

CONTENTS

FRANK H. J. FIGGE. Fluorescence Studies on Cancer. I. Porphyrin Metabolism, Harderian Gland Fluorescence, and Susceptibility to Carcinogenic Agents 465

EDWARD G. JONES, FRANK H. J. FIGGE, and J. MASON HUNDLEY, JR. Fluorescence Studies on Cancer. II. The Red Fluorescence of the Genitalia of Women 472

FRANK H. J. FIGGE, EDWARD G. JONES, and GERALDINE F. WOLFE. Fluorescence Studies on Cancer. III. The Extraction and Identification of Porphyrins from the Red-Fluorescent Exudates on the Genitalia of Women 483

OTTO ROSENTHAL and DAVID L. DRABKIN. The Oxidative Response of Normal and Neoplastic Tissues to Succinate and to *p*-Phenylenediamine 487

GLADYS CAMERON, C. J. KENSLER, and ROBERT CHAMBERS. The Action of Heptanal Sodium Bisulfite Methylsalicylate and of 2,4,6-Trimethylpyridine on Tissue Cultures of Human and Mouse Carcinoma and Rat Lymphosarcoma 495

J. F. RILEY and F. PETTIGREW. Retarding Effect of Glyceraldehyde on Benzpyrene Sarcoma Formation in Mice 502

R. M. MULLIGAN. Multiple Primary Tumors in Dogs 505

RIGOBERTO IGLESIAS, ALEXANDER LIPSCHÜTZ, and GUILLERMO NIETO. Antifibromatogenic Effects Produced by the Intermittent Action of Progesterone 510

CHRISTIANE DOSNE. Inactivation of Antifibromatogenic Substances (Progesterone and Desoxycorticosterone Acetate) in the Liver 512

ALEXANDER BRUNSCHWIG and THOMAS F. THORNTON, JR. An Experimental Study of the Lateral Spread of Epidermoid (Squamous Cell) Carcinoma in Man, and the Reaction of Such a Lesion to the Wound-Healing Stimulus 515

ABSTRACTS 519-528
Experimental Research, Animal Tumors 519-523
Clinical and Pathological Reports 523-528

THE OFFICIAL ORGAN OF THE
AMERICAN ASSOCIATION FOR CANCER RESEARCH, INC.

CANCER RESEARCH

This journal is sponsored by the American Association for Cancer Research, Inc., The Anna Fuller Fund, The International Cancer Research Foundation, and The Jane Coffin Childs Memorial Fund for Medical Research.

Advisory Board

MILDRED W. S. SCHRAM, *Chairman*

S. BAYNE-JONES
C. C. LITTLE
JAMES B. MURPHY
GEORGE M. SMITH

Editorial Committee

JAMES B. MURPHY, *Chairman*
WM. H. WOGLOM, *Secretary*
CLARA J. LYNCH, *Editor, Abstracts Section*

JOHN J. BITTNER
ALEXANDER BRUNSCHWIG
E. V. COWDRY
LOUIS I. DUBLIN
GIOACCHINO FAILLA
LOUIS F. FIESER
JACOB FURTH
WILLIAM U. GARDNER
JESSE P. GREENSTEIN
FRANCES L. HAVEN
BALDUIN LUCKÉ
E. C. MACDOWELL
G. BURROUGHS MIDER
EDGAR G. MILLER
JOHN J. MORTON
EDITH H. QUIMBY
MURRAY J. SHEAR
HAROLD L. STEWART
GRAY H. TWOMBLY
SHIELDS WARREN

Abstractors

W. A. BARNES
S. BAYNE-JONES
M. BELKIN
J. J. BITTNER
E. BOYLAND
J. B. BRIGGS
R. BRIGGS
W. J. BURDETTE
A. CLAUDE
P. P. COHEN
A. CORNELL
H. G. CRABTREE
H. J. CREECH
M. R. DEDDISH
T. B. DUNN
M. DURAN-REYNALS
M. J. EISEN
JOHN FOSTER
S. A. GRABER
W. E. GYE
A. HADDOW
J. B. HAMILTON
F. L. HAVEN
I. HIEGER
G. H. HOGEBOOM
M. E. HOWARD
R. N. JONES
E. L. KENNAWAY
J. G. KIDD
A. KIRSCHBAUM
E. A. LAWRENCE
R. J. LUDFORD
V. F. MARSHALL
W. V. MAYNEORD
J. L. MELNICK
C. J. MILLER
C. A. PFEIFFER
K. R. PORTER
E. H. QUIMBY
E. C. RICHARDSON
D. SHEMIN
R. E. SNYDER
E. E. SPROUL
K. G. STERN
C. WARREN
F. L. WARREN
H. Q. WOODARD
G. W. WOOLLEY

Published by The International Cancer Research Foundation.
Publication Office, 1500 Greenmount Ave., Baltimore 2, Maryland.

The annual subscription rates for one volume are: To members of the American Association for Cancer Research, Inc., $5.00; to others and to libraries, institutions, and organizations, $7.00. Business communications, remittances, and subscriptions should be addressed to Robert W. Briggs, Business Manager, 1500 Greenmount Ave., Baltimore 2, Md., or 2500 Lincoln-Liberty Building, Philadelphia 7, Pa.

No responsibility is accepted by the Committee, by the Board, or by the Publishers of *Cancer Research* for opinions expressed by contributors.

Entered as second class matter February 12, 1941, at the Post Office at Baltimore, Md., under the Act of March 3, 1879.

Copyright, 1944, by The International Cancer Research Foundation.

SEE INSIDE BACK COVER FOR INFORMATION FOR CONTRIBUTORS

CANCER RESEARCH

A MONTHLY JOURNAL OF ARTICLES AND ABSTRACTS REPORTING CANCER RESEARCH

VOLUME 4 AUGUST, 1944 NUMBER 8

Fluorescence Studies on Cancer*

I. Porphyrin Metabolism, Harderian Gland Fluorescence, and Susceptibility to Carcinogenic Agents

Frank H. J. Figge, Ph.D.

(From the Department of Anatomy, University of Maryland, School of Medicine, Baltimore 1, Maryland)

(Received for publication March 18, 1944)

It is well known that different species (8, 14, 35, 36, 44) and different organs and tissues (45) vary greatly in their susceptibility to carcinogenic agents. The only animals that have been extremely susceptible to the induction of tumors by means of physical and pure chemical agents are mice and rats. It has been found difficult and frequently impossible to produce tumors by the same methods even in other rodents (36). Shear (36) lists several investigators who found rabbits and guinea pigs refractory to the action of carcinogens that are potent in mice. By the administration of relatively tremendous quantities of methylcholanthrene, tumors have been induced in cottontail rabbits (39). Shimkin and Mider (37) induced tumors in a relatively high percentage of guinea pigs by subcutaneous injection of large doses of methylcholanthrene. From a tabulation of their own results and those of others (23, 33, 42) they state that, ". . . the relative carcinogenic potency of 20-methylcholanthrene, 3,4-benzpyrene, and 1,2,5,6-dibenzanthracene in guinea pigs is the same as in mice and rats (Fieser) but that guinea pigs are more resistant to the induction of subcutaneous neoplasms with these agents." Monkeys continue to resist the most drastic treatments with chemical carcinogens (1) and estrogenic compounds (1, 13); in the human subject, 3,4-benzpyrene has been used to treat 22 spontaneous neoplasms and 7 cures were described (2).

It has been postulated that certain unique features in the porphyrin metabolism of rats and mice are responsible for the extreme sensitivity of these animals to carcinogenic agents. The early observations (18) relating porphyrin metabolism and red fluorescence of the harderian gland to cancer susceptibility were limited to mice of inbred strains and of known susceptibility to spontaneous mammary carcinoma. There was some indication that the protoporphyrin 9 (type III) and coproporphyrin type I, which were responsible for the red fluorescence, were not formed in but merely excreted by the harderian gland (9, 21). The excess porphyrins excreted by the harderian glands were presumably produced in, or accumulated in, the mammary glands, lungs, skin, or subcutaneous tissues, which are most susceptible to spontaneous tumors.

The material included in the present report resulted from a study of the excretion of porphyrins by the harderian glands in other rodents and a number of other mammals. This investigation revealed that only the animals most susceptible to experimentally induced cancer (mice, rats, and hamsters) have red-fluorescent harderian glands that excrete porphyrins. The possibility that porphyrins of metabolic, parasitic, or chemotoxic origin are related to the high cancer incidence in certain organs and tissues in the human subject has also been examined. These studies have involved collaboration with clinical investigators and the results will be published in a series of reports. This first paper of the series will therefore include a comprehensive discussion of the hypothesis, a comparative study of harderian gland fluorescence and cancer susceptibility, and an outline of the general trend of the several investigations to be reported.

According to the hypothesis, cells and tissues subjected to abnormally high (optimum for sensitization) concentrations of certain porphyrins become hypersensitive to the action of normal as well as abnormal growth-stimulating factors or to relatively weak, natural, or artificial carcinogenic stimuli. The specific porphyrins that appear to have such a sensitizing

* This work was aided by grants from The Anna Fuller Fund and The International Cancer Research Foundation.

action are protoporphyrin 9 (type III) and coproporphyrin type I, but perhaps other porphyrins or their pyrrol degradation products may have a similar influence. These substances may all be derived from abnormal decomposition of hemoglobin, catalase, cytochrome *c*, or other heme substances. An interference with the normal synthesis of these heme compounds would also result in an accumulation of excess porphyrins. For additional data on normal and abnormal origin and metabolism of porphyrins the reader is referred to reviews of this subject (7, 11, 21, 41, 43). These sensitizing pyrrol compounds may exert their influence either within the cells where they arise or on the cell surface of other cells after diffusion or transportation.

One line of investigation that has been followed in the studies to be reported has been directed toward a search for the presence of abnormally high concentrations of porphyrins in the organs, tissues, and species that exhibit a high cancer incidence. This line of investigation has disclosed some general relationships that have been summarized as follows:

1. Protoporphyrin 9 (type III) and coproporphyrin type I have been found in abundance in young or rapidly growing, but not in mature non-growing tissues. This evidence was derived from the literature on porphyrins.

2. The porphyrin metabolism, as indicated by the fluorescence of the harderian gland, varied greatly in rodents and other orders of mammals. There appeared to be a definite correlation between the excretion of excess protoporphyrin 9 and coproporphyrin I and the susceptibility to experimentally induced cancer. The areas where these excreted porphyrins accumulated in and on the skin of the animal (Plate I) coincided with the areas that, according to numerous investigators (3, 4, 26, 28), gave rise to tumors when the entire animal was irradiated with ultraviolet light.

3. In the human subject certain sebaceous glands excrete protoporphyrin 9 and coproporphyrin I, the same porphyrins as those excreted by the harderian glands of some rodents. The areas of the skin where the red-fluorescent sebaceous secretions are most commonly found correspond to the areas where the incidence of skin cancer is highest.

4. Red-fluorescent exudates were found to occur frequently and intermittently on the cervix of the uterus and other parts of the genitalia of women (29). It has been shown by extraction and analysis that the red fluorescence of these exudates was due to the presence of protoporphyrin 9, small amounts of coproporphyrin, and other porphyrins (19). Most of the evidence indicates that these porphyrins resulted from decomposition of blood.

5. Some of the pathogenic bacteria and numerous saprophytic bacteria and molds produce porphyrins that may be partly responsible for the high cancer incidence in the areas where these organisms exist over long periods of time.

There is no evidence to indicate that abnormally high concentrations of porphyrins have a carcinogenic action. The porphyrins appear to sensitize the cells to the action of carcinogenic or growth stimuli that would not induce atypical growth in the absence of porphyrin sensitization. As sensitizing agents to carcinogenic stimuli, it is not necessary for the excess porphyrins to persist after the malignant transformation has occurred. No systematic attempt has been made to demonstrate porphyrins in neoplastic tissues but fluorescence examinations have shown porphyrin to be present in some neoplasms (chloroma, necrotic centers of tumors, and on the surface of necrotic exudates of cervical carcinomas). Most of the malignant tumors that have been examined were not red-fluorescent and the porphyrin content was not determined.

METHODS AND MATERIALS

The observations on the harderian gland fluorescence have extended over a period of three years and were carried out on a very heterogeneous group of animals. These were usually discarded animals or animals killed at the termination of other experiments. The routine procedure consisted in the removal and examination of the orbital contents in a darkened room to detect red-fluorescent glands or tissues. More specific details of the apparatus, conditions, and technic of such examinations have been described previously (16, 18).

The following animals were examined: 1,245 mice, 368 rats, 20 guinea pigs, 18 rabbits, 15 cats, 12 dogs, 12 chickens, 6 bats, 6 monkeys, 5 hamsters, 4 pigs, 3 squirrels, 2 opossums, 2 turtles, 2 cows, 2 alligators, 1 raccoon, 1 fox, 1 rattlesnake. The data on the fluorescence of the orbital contents and other organs of 8 human subjects examined at necropsy have been included.

The cancer susceptibility of some of the animals was estimated from a summarized tabulation of the data in Hartwell's survey of compounds (25) that have been tested for carcinogenic activity (Table II). The data that did not state the definite number of animals treated or the number of tumors induced were excluded. The table was then used to compare the harderian gland fluorescence with cancer susceptibility. Andervont's data on latent periods of induced tumors in mice, which were summarized by Fieser (14), were also used to estimate susceptibility in a few strains of mice, and the data of Burdette and

A. B.

Fig. 1.

Fig. 2.

A. B.

PLATE I *

FIG. 1, A and B—Illumination—visible light.

A—Normal rat with harderian gland exposed by displacement of the eyeball.
B—Rat maintained on a diet deficient in pantothenic acid. Note the accumulation of material resembling dried blood on nose and eye regions.

FIG. 2, A and B—Same as 1 A and B, but with near-ultraviolet illumination.

A—Note the red fluorescence of the harderian gland.
B—In the deficient rat, the porphyrins excreted by the harderian gland had passed to the nose via the naso-lacrimal duct. In deficient rats, porphyrins have a tendency to accumulate on the ears, eye region, nose, and forepaws which are the areas susceptible to induction of tumors by ultraviolet light. Some of this red-fluorescent material is frequently smeared on the same areas of the normal rat, but the amount is usually not sufficient to be seen in a fluorescence photograph.

* Permission to reproduce this illustration, which was previously published (17) in the *Journal of Laboratory and Clinical Medicine* (C. V. Mosby Co.), is gratefully acknowledged.

Strong (6) were employed to indicate the susceptibility of mice of C3H and JK strains.

RESULTS

Red-fluorescent porphyrin-excreting glands were found in the orbital cavities of all rats, all young mice, and some old mice. Derrien and Turchini (10) had described the red fluorescence of the harderian gland in these rodents of the genus *Mus* as early as 1924. Well-developed harderian glands were found in rabbits and guinea pigs but these were white-fluorescent or pale tan-fluorescent. The lacrimal glands and orbital tissues of the various other reptiles, birds, and mammals mentioned above were not red-fluorescent.

The most intense red fluorescence of the harderian gland was seen in the rat. In the hamsters, another red fluorescence at variable ages (between 200 and 300 days), while this persisted in the mice of the mediate red fluorescence (Table I). In the mice of certain strains (C57, JK, and I) the glands lost the

TABLE I: HARDERIAN GLAND FLUORESCENCE AND SUSCEPTIBILITY TO METHYLCHOLANTHRENE INDUCED TUMORS

Strain	Average intensity of red fluorescence of harderian gland		Latent period in weeks, MCA induced tumors		
	Young mice	Old mice	2 mg.*	1 mg.†	0.8 mg.*
C3H	++++	+++	8.5	9.8	9.6
A	+++	++	10.5		14.0
I	++	+	16.2		
JK	+			20.5	
C57	+				19.9

* Andervont's data from Fieser's review.
† Burdette and Strong's data.

TABLE II: THE COMPARATIVE SUSCEPTIBILITY OF ANIMALS TO THREE POTENT CARCINOGENIC HYDROCARBONS

Animal	1,2,5,6-Dibenzanthracene			3,4-Benzpyrene			20-Methylcholanthrene			Total		
	Number treated	Number tumors	Per cent	Number treated	Number tumors	Per cent	Number treated	Number tumors	Per cent	Number treated	Number tumors	Per cent
Mouse	11,593	4,553	39.3	6,020	2,621	43.5	3,346	1,563	46.7	20,959	8,737	41.7
Rat	1,412	314	22.2	2,991	1,107	37.9	454	177	39.0	4,857	1,598	32.9
Rabbit	265	20	7.5	238	27	11.4	26	0		529	47	8.9
Guinea pig	25	2	8.0	52	6	11.5	33	1	3.0	110	9	8.2
Fowl	234	57	24.3	50	6	12.0				284	63	22.2
Hamster				65	58	89.2				65	58	89.2
Newt	18	0		6	5	83.4				24	5	20.8
Frog	214	0		11	0		36	0		261	0	
Ferret	6	0		6	0					12	0	
Dog	1	0		6	0		6	0		13	0	
Monkey										?	0	
Man *				22	0					22	0	

* It should not be inferred from the data in this table that the human subject is resistant to carcinogenic agents. The 22 subjects tabulated were all patients with cancer. The benzpyrene was applied in these cases to treat the cancer and not to induce cancer. Two tumors have been recorded in laboratory workers who handled benzpyrene and methylcholanthrene, respectively. Other investigators deliberately painted 26 patients with benzpyrene daily for periods up to 120 days. The "precancerous changes" that developed retrogressed within 2 months after the cessation of treatment. (See Cook, J. W., and Kennaway, E. L., Am. J. Cancer, vol. 39, 1940, for original references.)

rodent though not a member of the same genus as mice and rats, red-fluorescent harderian glands were also observed. It was evident that the intensity of the red fluorescence was less than in the rat, and approximately equal to that found in the susceptible C3H mouse.

The red fluorescence of the harderian gland of mice of several strains has been described previously (18). The observations in this series were limited to mice of the C3H, A, JK, I, and C57 strains, which were the progeny of mice obtained from the colony of L. C. Strong. The harderian glands of immature mice from all strains were not red-fluorescent. The harderian glands from all strains developed red fluorescence at about the time the mice matured. The glands from mice of the C3H strain showed the maximum intensity and from C57 the least intense red fluorescence, while those from mice of the other strains exhibited inter-

C3H and A strains. The extraction of some of the non-red-fluorescent glands showed that porphyrins were present, but the concentration was not high enough to be detected by fluorescence in near ultraviolet light. Table I also shows that the latent periods for methylcholanthrene tumors are much shorter for the mice whose harderian glands contain the most porphyrin.

A tabulation of the susceptibility of animals to the action of three potent carcinogenic compounds (Table II) shows that mice and rats are most susceptible to these agents. The table also shows that they are the animals of choice for testing carcinogenic chemicals, probably because of their high susceptibility. Hamsters have not been used extensively, but the data in the table indicate that they are as susceptible as mice to methylcholanthrene tumors. This extreme susceptibility of the hamster was observed by Gye and Foulds

(22) and also by Halberstaedter (24). The consideration of their data stimulated the examination of the hamster. The harderian gland of the hamster was found to be red-fluorescent, hence it may be stated that the three animal species that have been found most susceptible to chemical carcinogens all have red-fluorescent, porphyrin-excreting harderian glands.

Table II indicates that mammals other than rodents have not been frequently selected to test carcinogenic hydrocarbons. The table is probably misleading in this respect, because many of the experiments that gave negative results were probably not reported. Cats and monkeys are not even mentioned, but monkeys have undoubtedly been given massive doses of one of or all these three compounds (1).

Susceptibility to the production of experimental neoplasia by ultraviolet irradiation has been reviewed by Blum (4), who states that mice and rats are the only animals that have responded with tumors. Rabbits and guinea pigs, whose harderian glands are not red-fluorescent, are refractory. Even prolonged irradiation of rabbits did not induce tumors (32), whereas the same investigators readily produced tumors in A and C strain albino mice. As C57 mice required more irradiation these data show that the animals most susceptible to the induction of tumors by light had red-fluorescent harderian glands.

Various factors such as pigmentation or thickness of the hair or epidermis have been advanced (4, 26, 27, 28) to account for the observed differences in susceptibility and the sites of development of tumors. These are all logical suggestions and the influence of these factors cannot be denied. However, a study of the porphyrin metabolism of the rat and the ultimate fate of the porphyrin secreted by the harderian gland (Plate I) shows that these porphyrins could be potent factors in determining the sites where tumors develop in rodents irradiated with ultraviolet light. Most investigators have observed that ultraviolet light induces malignant transformations on the ears, nose, forepaws, and about the eyes (3, 4, 26, 27, 28, 31); even depilated areas do not respond so readily as these sites. It may be seen (Plate I) that the areas where porphyrins from the harderian gland have a tendency to accumulate on the skin are precisely those that develop tumors when these animals are irradiated. According to Roffo (31), the forepaws of rats frequently develop tumors. The hair, pigment, and epidermal thickness are essentially the same on forepaws and hindpaws, but the forepaws (Plate I) are exposed to much more porphyrin from the harderian glands because they are used to wash the face with saliva and they thereby spread the porphyrins from the mouth and nose to the ears and other parts of the head.

The porphyrin-encrusted animal in the illustration had been fed a diet deficient in pantothenic acid (17). This caused changes in water metabolism (15) and decreased the production of saliva, so that the excreted porphyrin accumulated and became more abundant on the nose; in the normal rat this would have been smeared about on the ears and head. There is actually much more porphyrin on the ears and head of the vitamin-deficient rat than appears in the fluorescence photograph, because the intense blue-white fluorescence of thick fur dominates the picture except where the porphyrins are most abundant. This is also true of the normal rat, hence it is only occasionally that porphyrins become so abundant on the ears, nose, and forepaws as to fluoresce intensely enough to predominate over the fluorescence of the fur in these regions. For this reason the photograph of the vitamin-deficient rat, in which there was admittedly an exaggeration of the normal condition, was used to illustrate this point. Porphyrin encrustations on the nose have also been produced by water deprivation (15). Even the small amounts observed on the ears, eyes, nose, and forepaws of normal animals may be sufficient to sensitize the cells in these areas to the carcinogenic action of ultraviolet light and determine the site of neoplasia.

A subsidary experiment was performed to determine the fate of porphyrin injected into mice and rats. It was found that when these animals were injected with hematoporphyrin or protoporphyrin 9, these substances accumulated in the skin and subcutaneous tissues so that the entire shaved animal fluoresced red. In the injected unshaved animals only the skin of the ears, nose, eyelids, feet, and tail were red-fluorescent. With the exception of the hind feet, these are the areas most susceptible to the induction of tumors with light. Injected porphyrins also increased the intensity of red fluorescence of the harderian gland. These experiments demonstrate that if porphyrins were produced in excess in any tissue or organ of the rat they would become abundant in the skin, subcutaneous tissues, and harderian gland. Furthermore, the fact that these substances fluoresced when the animal was irradiated with near ultraviolet light indicates that they accumulated at depths that were reached by the wave lengths that incite fluorescence. Two possibilities, therefore, exist with regard to the site of action of the porphyrins that appear to sensitize mice and rats to light carcinogenesis: (a) sensitization may be due to the presence of porphyrins smeared on externally as the result of spreading harderian gland secretion, or (b) the sensitization may be related to the synthesis or accumulation of porphyrins in the skin and subcutaneous tissues of these rodents.

DISCUSSION

On the basis of red fluorescence alone Körbler (30) stated that "hematoporphyrin," acting as a photodynamic sensitizer, was an etiological factor in the production of cancer of the skin in the human subject. This view developed from an analysis of data on 238 cases of skin cancer. The figures show that 97.5 per cent of the skin tumors developed at sites exposed to the sun. Only 66 per cent of these occurred in the group of farmers and sailors, which composed 90 per cent of the population. In other words, the 10 per cent of the population that were not exposed to the sun had a disproportionally high cancer incidence. The attempt to explain this on the basis of hematoporphyrin sensitization has been justly and unjustly criticised by several investigators (4, 28). The criticism that red fluorescence is not a satisfactory proof of hematoporphyrin (28) is valid for any porphyrin. This hypothesis, which resembles the one that has been developed in this work, is extremely interesting, but it is unfortunate that the red fluorescence was ascribed to hematoporphyrin because this porphyrin forms only under artificial conditions (11, 21).

Hematoporphyrin has also been used to enhance tumor formation in mice by light (5), but other investigators (40) observed no conclusive influence on the rate of induction of benzpyrene tumors in irradiated mice. Mice and rats would not be appropriate animals to use for testing the sensitizing influence of porphyrins or other sensitizing agents because these animals manufacture and excrete excess porphyrins. In this connection it is of interest that the administration of 1,2,5,6-dibenzanthracene to rabbits results in urinary excretion of abnormal amounts of coproporphyrin I (11). Coproporphyrin I and protoporphyrin 9 have little photosensitizing action (21) as compared with hematoporphyrin. However, one or both of the former may be more potent in sensitizing cells to the carcinogenic influence of light or chemical carcinogens.

The constant and simultaneous occurrence of pairs of porphyrins was referred to by Fischer (21) as the dualism of the porphyrins. It is generally assumed that this dualism is the result of a necessarily mixed synthesis. Coproporphyrin I according to this concept is a coincidental, nonfunctional by-product of protoporphyrin synthesis. It may well be that coproporphyrin I does have some function to perform. Either coproporphyrin I alone or the ratio of protoporphyrin 9 and coproporphyrin I may regulate the mechanisms that initiate cell division and growth. For a more extensive discussion of the normal occurrence, abundance, and fate of these two porphyrins in living tissues than that which follows, the reader is referred to comprehensive reviews (11, 21, 34, 41, 43) in which the original references have been given.

Metal porphyrin protein complexes such as hemoglobin, chlorophyl, myoglobin, cytochrome *c*, catalase, and peroxidase occur in practically all cells. Free protoporphyrin 9 and coproporphyrin I are, however, not so abundant in most cells, though commonly found in young tissues and in cells undergoing rapid division. In vertebrates in general, and mammals and man in particular, there is a considerable body of evidence to support the view that protoporphyrin 9 and coproporphyrin I are formed as intermediates in the synthesis of hemoglobin (11, 21, 34). However, these substances are not formed in the normal degradation of hemoglobin (11, 34). If the other iron porphyrin substances (myoglobin, cytochrome *c*, etc.) normally found in many cells undergo similar degradation, the intermediate decomposition products are not known. Porphyrins may be formed in this process, but even so the amount from this source would be relatively small.

The most striking example of the association of high concentrations of protoporphyrin 9 and coproporphyrin I with the tissues and cells that are actively growing and dividing is found in the bone marrow (34). Here the rate of cell division is higher throughout life than in any other organ or tissue in the body. Amniotic fluid, developing embryos, and chick embryos contain protoporphyrin in relatively large amounts (34). Other areas where porphyrins and rapid mitosis occur coincidently are the base of hair follicles, the skin, growing cartilage, and ossifying tissues (20). Rapidly growing organisms such as yeasts and bacteria produce relatively large quantities of protoporphyrin 9 and coproporphyrin I (21).

The porphyrins present in tissues that are actively growing and dividing are probably synthesized within the cells. The association of protoporphyrin 9 and dividing cells may be coincidental and depend upon the necessity of supplying the newly formed cells with the required complement of iron-porphyrin-enzymes that are not later replaced or, if so, only slowly. The rapid synthesis of porphyrin in the cell would thus not occur until cells prepare to undergo division. On the other hand, protoporphyrin 9 and coproporphyrin I synthesis may be speeded up in the cell in an attempt to compensate for an inadequate metabolic mechanism. The inadequate metabolic mechanism postulated as a primary stimulus to porphyrin formation may be the result of (a) changes in the size of the cells that disturb the normal ratio of cell surface and volume; (b) the destruction, inactivation, or deficiency of heme or non-heme respiratory enzymes; (c) an intracellular parasite such as a virus that may require the protein or heme component

of a respiratory enzyme such as cytochrome *c* for its continued existence. With reference to the second and third possibilities just stated, a deficiency of cytochrome *c* (12) and other cytochromes (38) has been demonstrated in various malignant tissues. There are numerous other mechanisms that might interfere with the cellular metabolism and cause increased production or accumulation of protoporphyrin 9 and coproporphyrin I. According to the hypothesis, these excess porphyrins would then sensitize the cells to the action of normal as well as abnormal growth-stimulating factors or relatively weak carcinogenic agents.

SUMMARY

(a) The examination of numerous birds, reptiles, and mammals showed that red-fluorescent harderian glands were present only in mice, rats, and hamsters. The data in the literature indicate that of all the animals tested, these three species are the most susceptible to induction of tumors by carcinogenic agents. Since the red-fluorescent porphyrin-excreting harderian gland reflects the porphyrin metabolism of other organs and tissues, a relationship between excess porphyrins (or a unique porphyrin metabolism) and susceptibility to carcinogenic agents is postulated. Protoporphyrin 9 and coproporphyrin I are the specific porphyrins excreted by the harderian glands.

(b) When porphyrins were injected into the peritoneal cavity of rats, these substances soon became concentrated in the skin and subcutaneous tissues and the harderian glands. A study of the ultimate fate of the porphyrins excreted by the harderian glands shows that they are smeared on the areas of the skin of mice and rats where tumors develop when these animals are irradiated with ultraviolet light. Here, too, the only animals that have been found susceptible to the induction of tumors by ultraviolet light are mice and rats, which have red-fluorescent (porphyrin-excreting) harderian glands. These data support the original hypothesis that there is a direct or an indirect relationship between porphyrins or porphyrin metabolism and cancer susceptibility.

REFERENCES

1. Allen, E. Report of Activities during 1940 (p. 8). Report of Activities during 1941 (p. 24). International Cancer Research Foundation.
2. Bauer, K. H. Fortschritte der experimentellen Krebsforschung. Arch. f. klin. Chir., **189**:123-184. 1937.
3. Beard, H. H., Boggess, T. S., and von Haam, E. Experimental Production of Malignant Tumors in the Albino Rat by Means of Ultraviolet Rays. Am. J. Cancer, **27**: 257-266. 1936.
4. Blum, H. F. Sunlight and Cancer of the Skin. J. Nat. Cancer Inst., **1**:397-421. 1940.
5. Büngeler, W. Über den Einfluss photosensibilisierender Substanzen auf die Entstehung von Hautgeschwülsten. Ztschr. f. Krebsforsch., **46**:130-167. 1937.
6. Burdette, W. J., and Strong, L. C. The Inheritance of Susceptibility to Tumors Induced in Mice. I. Tumors Induced by Methylcholanthrene in Five Inbred Strains of Mice. Cancer Research, **3**:13-20. 1943.
7. Carrié, C. Die Porphyrine. Leipzig: G. Thieme. 1936.
8. Cook, J. W., and Kennaway, E. L. Chemical Compounds as Carcinogenic Agents. First Supplementary Report: Literature of 1937. Am. J. Cancer, **33**:50-97. 1938.
9. Derrien, E. Note préliminaire sur quelques faits nouveaux pour l'histoire naturelle des porphyrines animales. Compt. rend. Soc. de biol., **91**:634-636. 1924.
10. Derrien, E., and Turchini, J. Sur l'accumulation d'une porphyrine dans la glande de Harder des Rongeurs du genre *Mus* et sur son mode d'excrétion. Compt. rend Soc. de biol., **91**:637-639. 1924.
11. Dobriner, K., and Rhoads, C. P. The Porphyrins in Health and Disease. Physiol. Rev., **20**:416-468. 1940.
12. Du Bois, K. P., and Potter, V. R. Biocatalysts in Cancer Tissue. I. Cytochrome c. Cancer Research, **2**:290-293. 1942.
13. Engle, E. T., Krakower, C., and Haagensen, C. D. Estrogen Administration to Aged Female Monkeys with No Resultant Tumors. Cancer Research, **3**:858-866. 1943.
14. Fieser, L. F. Carcinogenic Activity, Structure, and Chemical Reactivity of Polynuclear Aromatic Hydrocarbons. Am. J. Cancer, **34**:37-124. 1938.
15. Figge, F. H. J., and Atkinson, W. B. Relation of Water Metabolism to Porphyrin Incrustations in Pantothenic Acid-Deficient Rats. Proc. Soc. Exper. Biol. & Med., **48**: 112-114. 1941.
16. Figge, F. H. J. Near-Ultraviolet Rays and Fluorescence Phenomena as Aids to Discovery and Diagnosis in Medicine. Bull. Univ. Maryland School Med., **26**:165-176. 1942.
17. Figge, F. H. J., and Salomon, K. Prevention of Porphyrin Incrustations on Pantothenic Acid-Deficient Rats by Harderian Gland Ablation. J. Lab. & Clin. Med., **27**: 1495-1501. 1942.
18. Figge, F. H. J., Strong, L. C., Strong, L. C., Jr., and Shanbrom, A. Fluorescent Porphyrins in Harderian Glands and Susceptibility to Spontaneous Mammary Carcinoma in Mice. Cancer Research, **2**:335-342. 1942.
19. Figge, F. H. J., Jones, E., and Wolfe, Geraldine F. Fluorescence Studies on Cancer. III. The Extraction and Identification of Porphyrins from the Red-Fluorescent Exudates on the Genitalia of Women. Cancer Research, **4**:483-486. 1944.
20. Figge, F. H. J. Unpublished Observations.
21. Fischer, H., and Orth, H. Die Chemie des Pyrrols. Vol. II, 1st half. Pp. 764. Leipzig: Akademische Verlagsgesellschaft m. b. H. 1937.
22. Gye, W. E., and Foulds, L. A Note on the Production of Sarcomata in Hamsters by 3:4-Benzpyrene. Am. J. Cancer, **35**:108. 1939.
23. Haagensen, C. D., and Krehbiel, O. F. Liposarcoma Produced by 1:2-Benzpyrene. Am. J. Cancer, **27**:474-484. 1936.
24. Halberstaedter, L. Tumors Produced in Hamsters by Benzpyrene. Am. J. Cancer, **38**:351-358. 1940.
25. Hartwell, J. L. Survey of Compounds Which Have Been Tested for Carcinogenic Activity. Federal Security Agency, National Institute of Health, National Cancer Institute, United States Public Health Service, Bethesda, Maryland. 1941.
26. Hueper, W. C. Cutaneous Neoplastic Responses Elicited by Ultraviolet Rays in Hairless Rats and in Their Haired Litter Mates. Cancer Research, **1**:402-406. 1941.

27. Hueper, W. C. Morphological Aspects of Experimental Actinic and Arsenic Carcinoma in the Skin of Rats. Cancer Research, **2**:551-559. 1942.
28. Hueper, W. C. Occupational Tumors and Allied Diseases. Springfield, Ill.: Charles C. Thomas. 1942.
29. Jones, E. G., Figge, F. H. J., and Hundley, J. M., Jr. Fluorescence Studies on Cancer. II. The Red Fluorescence of the Genitalia of Women. Cancer Research, **4**: 472-482. 1944.
30. Körbler, J. Das Sonnenlicht in der Ätiologie der Hautkarzinome. Strahlentherapie, **52**:353-358. 1935.
31. Roffo, A. H. La etiología físico-química del cáncer en relación con las irradiaciones solares. Bol. Inst. de med. exper. para el estud. y trat. d. cancer, **13**:455-480. 1936.
32. Rusch, H. P., and Baumann, C. A. Tumor Production in Mice with Ultraviolet Irradiation. Am. J. Cancer, **35**: 55-62. 1939.
33. Schabad, L. M. On Experimental Tumors in Guinea Pigs. Arch. Biol. Sc. (U. S. S. R.), **51**:112-119. 1938.
34. Schultze, M. O. Metallic Elements and Blood Formation. Physiol. Rev., **20**:37-67. 1940.
35. Seelig, M. G., and Cooper, Z. K. A Review of the Recent Literature of Tar Cancer (1927-1931 Inclusive). Am. J. Cancer, **17**:589-667. 1933.
36. Shear, M. J. Studies in Carcinogenesis. V. Methyl Derivatives of 1:2-Benzanthracene. Am. J. Cancer, **33**:499-537. 1938.
37. Shimkin, M. B., and Mider, G. B. Induction of Tumors in Guinea Pigs with Subcutaneously Injected Methylcholanthrene. J. Nat. Cancer Inst., **1**:707-725. 1941.
38. Stern, K., and Willheim, R. The Biochemistry of Malignant Tumors. Brooklyn, N. Y.: Reference Press. 1943.
39. Syverton, J. T., Berry, G. P., and Dascomb, H. E. Studies on Carcinogenesis in Rabbits. I. Malignant Tumors Induced in Cottontail Rabbits by the Injection of Methylcholanthrene in Tricaprylin. Cancer Research, **2**:436-444. 1942.
40. Taussig, J., Cooper, Z. K., and Seelig, M. G. The Effect of Light on Benzpyrene Cancer in Mice. Surg., Gynec. & Obst., **66**:989-993. 1938.
41. Vannotti, A. Porphyrine und Porphyrinkrankheiten. Berlin: Julius Springer. 1937.
42. Warren, S., and Gates, Olive. Spontaneous and Induced Tumors of the Guinea Pig. Cancer Research, **1**:65-68. 1941.
43. Watson, C. J. The Pyrrol Pigments. Downey's Handbook of Hematology. Vol. **4**:2445. 1938. New York: Paul B. Hoeber, Inc. 1938.
44. Woglom, W. H. Experimental Tar Cancer. Arch. Path., **2**:533-576, 709-752. 1926.
45. Woglom, W. H. Agent and Soil in Experimental Carcinogenesis. Am. J. Cancer, **32**:447-448. 1938.

Fluorescence Studies on Cancer*

II. The Red Fluorescence of the Genitalia of Women

Edward G. Jones, M.D., Frank H. J. Figge, Ph.D., and
J. Mason Hundley, Jr., M.D.

(From the Departments of Gynecology and Anatomy, University of Maryland, School of Medicine, Baltimore 1, Maryland)

(Received for publication March 18, 1944)

Certain observations have given rise to the hypothesis that porphyrins may have a direct or indirect relationship to cancer susceptibility (9). These observations include: (a) A parallelism between the degree of susceptibility to spontaneous mammary carcinoma and the degree of red fluorescence of the harderian gland in mice has been demonstrated (9). The red fluorescence of the harderian gland in rodents has been shown to be related to the high protoporphyrin content (1, 8, 11, 19). (b) A survey of numerous rodents and other vertebrates has revealed that mice, rats, and hamsters are the only animals that possess porphyrin-excreting harderian glands (4). Furthermore, the relatively high susceptibility of mice and rats to the induction of tumors by means of pure carcinogenic hydrocarbons has been partly responsible for the fact that they have been most frequently chosen to test such agents (13, 16). Experiments with laboratory animals have yielded data indicating that protophorphyrin may directly sensitize cells to the action of methylcholanthrene (7).

It was next postulated that if any of the porphyrins are an etiological factor in human cancer it should be possible to demonstrate these red-fluorescent deposits on organs and tissues exhibiting a high cancer incidence. Since carcinoma of the cervix of the uterus is the most prevalent neoplasm in the women of this country (2, 14) we have investigated this structure and other parts of the female genitalia for red fluorescence. The observations in three types of patients will be described: (a) normal patients; (b) patients with inflammatory lesions or lesions believed to be precancerous; (c) patients with malignant tumors.

Red-fluorescent deposits were observed in variable amounts in some of the patients in each group though, to our knowledge, they have not been described previously. This is somewhat surprising in view of their frequent occurrence and the ease with which red-fluorescent areas and deposits could be demonstrated.[1] Our observations support the possibility that porphyrins may be one of the etiological factors in genital neoplasia in women, but conclusions regarding the significance of these observations must await further experimental analysis and this paper will be confined, therefore, to a simple description.

METHODS AND MATERIALS

The patients were seen in the University Hospital, the Baltimore City Tuberculosis Hospital, and the University Hospital Gynecological Clinic. Most of those selected for examination gave a history of an inflammatory lesion of the genital tract, or of a carcinomatous lesion, treated or untreated. The woman's age, color, chief complaint, the primary clinical diagnosis, cervical diagnosis, regularity and stage of menstrual cycle, significant family and past history, and the number of full term pregnancies, stillbirths, and abortions were recorded. A description of the size, shape, position, and the surface of the cervix was given, and the type and amount of exudate present in the vagina and on the cervix were listed for all patients examined with the speculum. Similarly, the fluorescence of the cervix, vagina, clitoris, labia minora, and labia majora was tabulated. The degree of intensity of red fluorescence was recorded in a semi-quantitative way, by means of a numerical scale. Thus, intense red fluorescence covering most of the organ examined was recorded as plus 4; small amounts were represented by plus 1; plus 2 and plus 3 were intermediate amounts. "Slight" was used to represent traces of red or orange-red fluorescence. Where indicated, a microscopic examination was made of the exudate and the pH determined. Any treatment,

* This work was aided by grants from The Anna Fuller Fund and The International Cancer Research Foundation.

[1] Since completion of this manuscript we have discovered that Hauser in 1929 reported observations on red fluorescence of the genitalia in women and postulated the presence of porphyrins. This work will be more fully discussed in paper III of this series.

douche, soap, lubricant, or powder used in the week preceding examination was also listed.

The examinations were made in a darkened room. A tungsten filament white light that could be turned on and off was used for the routine pelvic examinations. A mercury arc lamp with a mantle filter transmitting wave lengths between 3,100 A. and 4,000 A. was employed for detecting red fluorescence. The material and technic necessary for such examinations have been described in detail (3, 5). The vagina and cervix were exposed with a sterilized, nonlubricated bivalve speculum. When a slight or intense red-fluorescent exudate was observed it was removed with clean cotton swabs or clean cotton applicators. Intensely red-fluorescent swabs were saved for Kodachrome photography and identification of the porphyrins (6). Some of the representative red-fluorescent deposits were photographed in color *in situ*. This was accomplished by placing the patients on a cystoscopic table in the lithotomy position. They were thus immobilized to permit the necessarily long exposures (15 minutes). The other details of the technic have been described previously (5).

OBSERVATIONS

The first woman examined was a patient with a recurrent carcinoma of the cervix following radium treatment a year previously. Speculum examination showed a necrotic, ulcerated area around the external orifice. A moderate amount of purulent discharge was present in the vagina. Ultraviolet light showed an intense orange-red glow from the cervix (plus 3 or plus 4). Most of the fluorescent material could be wiped off and under white light proved to be necrotic material from around the external orifice. The clitoris also was orange-red-fluorescent.

The second patient seen was bleeding slightly from an incomplete abortion that had occurred a week previous to examination. Red fluorescence of plus 2 intensity was seen in the exudate on the cervix and vaginal wall.

A systematic survey was then started, in the course of which it was found in general that the intense red fluorescence found in the first 2 women was not observed in all cases or even in all patients with bleeding or with purulent discharges. In the majority no red fluorescence was observed; in these the labia and clitoris were nonfluorescent, the vagina was white-fluorescent and this white fluorescence extended over the cervix to its orifice. When blood was present it appeared black, because blood is not fluorescent. Mucus and purulent material were usually slightly white-fluorescent, surface lacerations nonfluorescent.

Examination of patients with general medical diseases was then initiated in the hope of discovering the source and significance of the red fluorescence. Among these, a colored woman, under observation for hyperthyroidism, exhibited brilliant red fluorescence of the clitoris, labia minora, and medial surface of the labia majora. She was menstruating, but there was no fluorescence of the vagina and cervix. The next day the fluorescent area was photographed on Kodachrome film (Figs. 4, 5). Then an attempt was made to wipe off the fluorescent material, but only a portion could be removed; it seemed to be in the epithelial layers. About 10 days later the developed films were found to be slightly overexposed. The patient was re-examined to see if repeat films could be taken, but the entire red-fluorescent material had disappeared except for a slight amount on the clitoris. Meanwhile she had been given thiouracil and a high vitamin diet supplemented with thiamine chloride, and also had taken daily soap baths. This patient was examined again 2 days after her next menstrual period began and there was no notable fluorescence. The day after cessation of the menses, intense red fluorescence reappeared on the clitoris, vestibule, and about the vaginal orifice.

Four untreated hyperthyroid patients were examined and their genitalia were not red-fluorescent. Many other patients had been examined, of whom some showed red fluorescence. A few of these were in the early postmenstrual part of their cycle, from which it was inferred that the red fluorescence was related to the porphyrins derived from the decomposition of blood in the menstrual fluid. To investigate this possibility the fate of the blood in lochia and menstrual fluid was determined.

Lochia pads were collected each morning from the obstetrical ward. The pads were examined under near ultraviolet light and some, showing intense red fluorescence, were saved for porphyrin analysis. After 1 week it was possible to summarize the findings (Table VI) as follows: Red fluorescence was usually not present until the serosal discharge began and it disappeared after a few days in some cases. In other women, large amounts persisted throughout confinement, while the pads of still other women did not show any fluorescence. The findings could not be related to sulfa drugs, stilbestrol, nursing, or ocytocics. The nurse in charge was then questioned about the postpartum course of each patient, and it was elicited that the lochia had been foul in nearly all the patients with definitely red-fluorescent pads. Examination of postpartum patients with foul lochia revealed plus 4 red fluorescence of the labia and clitoris under near ultraviolet light (Figs. 8, 9). This was occasionally present as long as 6 weeks postpartum.

The pads from 8 menstruating women were collected for 3 days; some showed small amounts of red fluorescence.

Controls (infants, girls, and men).—We noted in our clinic patients that many showed red fluorescence of the clitoris when no other fluorescence was present. To test whether or not this originated from menstrual blood, 62 male and female infants were examined. No red fluorescence other than small amounts from meconium was seen on the clitoris or penis. In addition, recently circumcised infants did not show fluorescence in the circumcision wound. Twelve girls between 1 and 10 years of age were examined and no definite red fluorescence was shown. However, when the glans penis in 14 male patients was examined, red fluorescence was seen in varying amounts on 4, chiefly along the dorsal surface near the corona. The patient who showed the most intense red fluorescence was an elderly man who had been confined to bed in the hospital for a year. No patient who had been circumcised showed fluorescent material. These observations indicate that the red-fluorescent material surrounding the clitoris may not be derived from blood.

Carcinoma Cases

One case of carcinoma of the clitoris was seen. The patient was a 76 year old white woman. The tumor replaced the clitoris and measured about 2 cm. in all diameters. Under near ultraviolet light there was intense orange-red fluorescence of the labia and especially of the prepuce adjacent to the lesion. Fluorescence of the lesion itself was less intense. Biopsy of the fluorescent portion of the prepuce failed to reveal any evidence of malignancy.

Three cases of carcinoma of the cervix recurring some months after radium treatment were examined. All showed some red fluorescence of the genitalia; 2 of the cervices were red-fluorescent. On the other hand, several cases of carcinoma of the cervix with complete gross regression following treatment were seen, and in most of these red fluorescence was absent. Six untreated cases of carcinoma of the cervix were examined; in 2 the genitalia showed no red fluorescence. These women were 62 and 33 years old respectively. The first examination of the latter patient was in the intermenstrual stage. She had been taking douches daily as well as on the morning of the examination. When she was re-examined near the end of her next menstrual period, intense red fluorescence was present on the labia and clitoris. The other carcinoma patients showed varying amounts of red fluorescence. One of these is of special interest because the red fluorescence was considerable, but a gross lesion was not visible on speculum examination. The patient was a 45 year old colored woman who was first seen complaining of lower abdominal pain and intermittent uterine bleeding. She had been treated elsewhere with radium 11 months before our examination for the bleeding, but had had a recurrence of the bleeding about 5 months later. Examination revealed the presence of foul-smelling blood in the vagina. When this was removed the cervix showed old lacerations; it was slightly enlarged and nearly in contact with the vault, but otherwise appeared normal. The uterus felt somewhat enlarged. The bloody discharge was nonfluorescent. There was some red fluorescence of the clitoris and labia minora. The diagnosis was deferred and she returned to the clinic in 1 week. Her menstrual bleeding had stopped 3 days previously. There was a profuse, foul-smelling, watery discharge in the vagina that emitted a brilliant red glow under near ultraviolet light. This was removed with cotton swabs, photographed (Figs. 6, 7), and saved for analysis (6). The material gave an acid reaction with litmus paper. Gram stain showed many gram-positive and gram-negative organisms. There were a few fibrous strands and old epithelial cells. No well-preserved white blood corpuscles were seen. The patient was instructed not to take any douches and to return 1 week later. The discharge at this time was even more copious, foul, and still retained the intense red fluorescence noted before. Urine studies were negative; hemoglobin was 60 per cent. The patient died before a diagnostic curettage could be obtained. At necropsy, an extensive adenocarcinoma of the cervix or corpus [2] was found arising above the external os and extending through the cervical canal to the internal os. Under near ultraviolet light there were a few spots of red fluorescence visible on the lower portion of the lesion (Figs. 1, 2, 3) that could be washed off.

Summary of Clinical Observations

The clinical material is summarized in the tables. One hundred and twenty-one women were examined, of whom 85 were colored. Red fluorescence was observed in 48 of the women. In Table I the cases

Table I: The Incidence of Red Fluorescence on the Genitalia of Women with Normal Cervices, Cervicitis, and Carcinoma of the Cervix

		Red fluorescence				
Cervix	Number of cases	Clitoris	Labia minora	Labia majora	Vagina	Cervix
Cervicitis	39	12	3	1	1	1
Normal	62	16	11	6	6	4
Carcinoma (untreated or recurrent)	9	6	5	5	4	4
Total	110	34	19	12	11	9

[2] Mucin stain to identify cervical glands was inconclusive.

are arranged according to the cervical diagnosis: "Normal," "Cervicitis," and "Carcinoma." Red fluorescence was seen in the vagina in 12 and on the cervix in 9 of the 121 women. There were 39 cases of cervicitis; 1 of the cervices was red-fluorescent. In 62 women, the cervix appeared normal; 4 of these were red-fluorescent. Of 9 cases of untreated or recurrent carcinoma of the cervix, the cervix was red-fluorescent in 4.

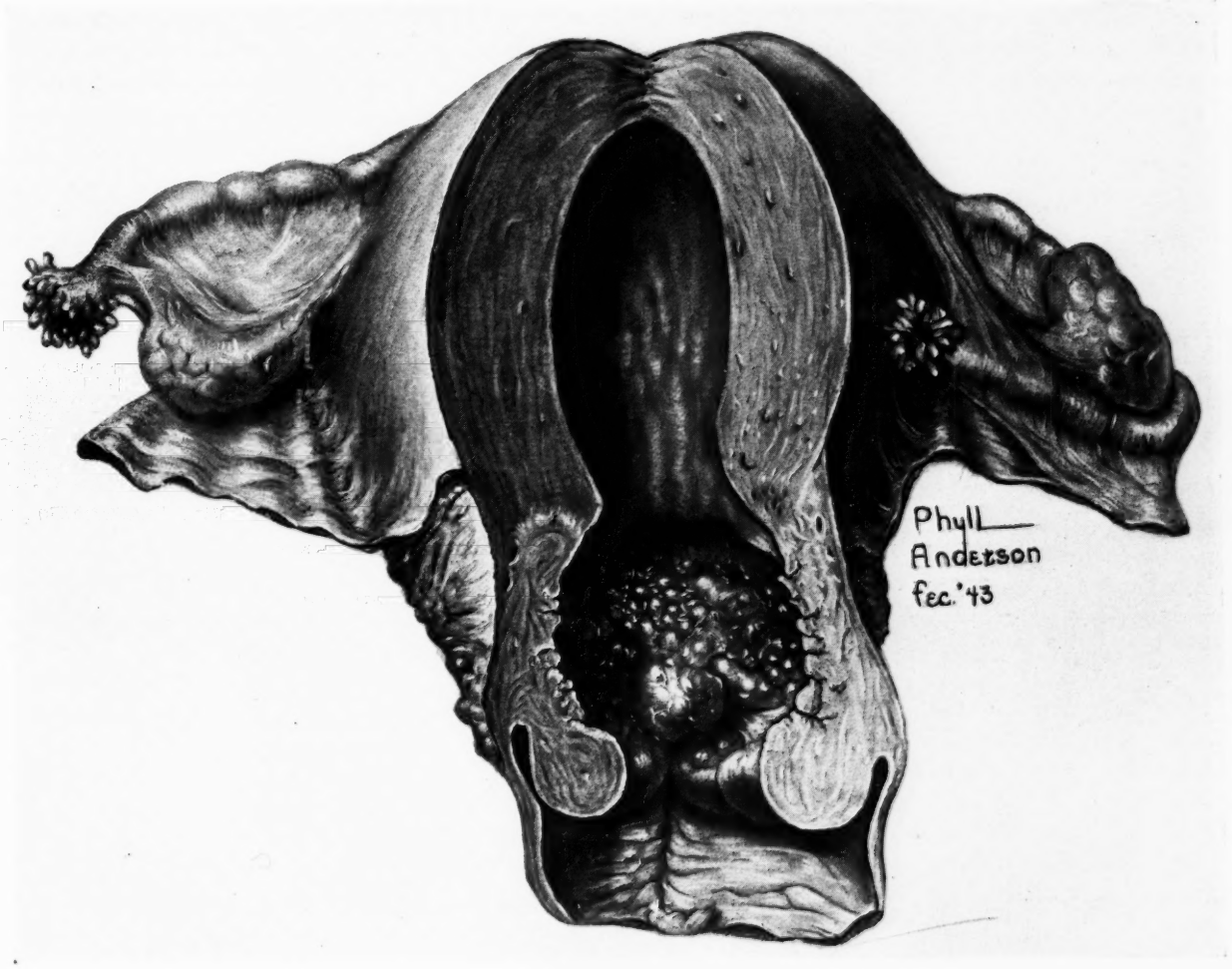


FIG. 1.—E. G. Adenocarcinoma of cervix or corpus uteri (mucin stains inconclusive) extending from above external os to about level of internal os.

Table II shows the relationship of the red fluorescence to the stage of the menstrual cycle. In 16 women seen during their menstrual period, or during the postmenstrual phase, red fluorescence was observed on the clitoris in 11, on the labia minora in 9, on the labia majora in 5, on the vagina in 4, and on the cervix in 4. Fluorescence at other phases of the cycle occurred less frequently. During pregnancy, red fluorescence was infrequent and was of low intensity when present. Red fluorescence of the clitoris was more common than at other sites, and when present in association with red fluorescence elsewhere it was usually the most intense. Red fluorescence of the clitoris was seen in 41 of the 121 patients. It was of plus 3 or plus 4 intensity in 11 of these. Five women showed a bluish-

TABLE II: THE INCIDENCE OF RED FLUORESCENCE ON THE GENITALIA OF WOMAN. INFLUENCE OF THE MENSES

STAGE OF MENSTRUAL CYCLE IN NONMALIGNANT CASES

		RED FLUORESCENCE				
	Number of cases	Clitoris	Labia minora	Labia majora	Vagina	Cervix
Menopausal †	34	4	2	2	2	0
Menstrual	6	5	3	3	2	2
Postmenstrual	10	6	6	2	2	2
Intermenstrual	28	8	2	2	1	2
Premenstrual	15	7	1	0	1	0
Stage unknown	6	1	0	0	0	0
Pregnant	10	3	2	0	0	0
*Postpartum (6 wks.)	2	0	0	0	0	0
Total	111	34	16	9	8	6

* About 15 other postpartum women were examined in the Obstetrical Clinic but are not included in the series.

† Includes postmenopausal cases.

white fluorescence of the clitoris. An exudate that was slightly white-fluorescent was often seen on the clitoris. Tables IV and V offer a more complete summary of most of those cases showing large amounts of red-fluorescent material on the labia, vagina, or cervix.

Table III lists the cases by principal diagnosis. They have been arbitrarily fitted into 17 diagnoses, each being given 1. Carcinoma cases have already been discussed; some of the untreated ones are given in detail in Table V. There were 7 cases of acute cervicitis. The smears from 3 of these were positive on gram stain for gonococci. In 1, the cervix was hypertrophied, eroded, and appeared granulomatous. Stain for gonorrhea, dark field examination, and serological tests were negative. Repeated biopsies showed acute and chronic inflammatory reaction without any evidence of malignancy. All these cases were accompanied by a purulent discharge, which was not red-fluorescent. About 35 women showed chronic infection of the cervix, but all except 5 could be placed under another diagnosis; the cervix of none of these 5 women was red-fluorescent. One of these was cauterized after the initial examination; inspection 2 weeks later failed to show any evidence of red fluorescence.

TABLE III: GENERAL SUMMARY OF RED FLUORESCENCE ON GENITALIA OF WOMEN

Diagnosis	Number of cases	RED FLUORESCENCE: Clitoris	Labia minora	Labia majora	Vagina	Cervix
Normal	13	3	2	3	4	2
Carcinoma of Cervix						
1) Untreated or recurrent	9	5	4	4	4	4
2) Treated, with cervix healed	8	3	1	1	0	0
Carcinoma clitoris, untreated	1	1	1	1	—	—
Pulmonary tuberculosis	14	7	1	1	0	0
Acute cervicitis	7	1	1	0	0	0
Chronic cervicitis	5	4	0	0	0	0
Pregnancy	10	2	0	0	0	0
Incomplete abortion	2	2	0	0	0	2
Syphilis, recent	2	1	0	0	0	0
Hyperthyroidism, pronounced	5	1	1	1	1	0
Fibromyomas	4	2	2	1	1	1
Amputation of cervix, recent	2	0	1	0	0	1
Metrorrhagia, unknown etiology	1	0	1	1	1	1
Draining cul-de-sac abscess	2	2	1	1	1	1
Atrophy of vulva or vagina and cervix, pronounced	4	0	0	0	0	0
Miscellaneous	31	7	1	0	1	0
Total	119	40	16	13	12	11

Two pregnant women were examined. All the trimesters were represented; 2 were in labor, in 1 of whom the membrances had ruptured before examination; the other had her membranes intact and the mucous plug was nonfluorescent. No red fluorescence was seen on any of these women except for moderate red fluorescence on the clitoris on the 2 women in labor. Two patients were seen bleeding slightly after an incomplete abortion. One of these showed red fluorescence of the cervix that was not present on re-examination 1 week later.

Two women had recently acquired syphilis, 1 with a freshly healed primary chancre on the labia minora, the other with a condyloma latum on the left labia minora. The latter lesion measured about 4 cm. in diameter and contained spirochetes. Neither patient showed fluorescence of the labia, vagina, or cervix.

Four women had fibromyomas of the uterus large enough so that they could be diagnosed with certainty on examination. Only 1 of these showed notable red fluorescence of the vagina, cervix, and labia. Two

DESCRIPTION OF FIGURES 2 TO 9 *

FIG. 2.—E. G. (See Fig. 1) Cervix uteri replaced by adenocarcinoma as seen in ordinary light.

FIG. 3.—E. G. (Same region as Fig. 2) Cervix uteri in near ultraviolet light. Small areas of red-fluorescent material on surface of adenocarcinoma.

FIG. 4.—L. H. (See Table IV) Vulva and vagina as seen in ordinary light. Note pink color of vagina and bright red menstrual blood.

FIG. 5.—L. H. (Same patient as Fig. 4) Vulva and vagina as seen in near ultraviolet light. Film slightly over exposed, but shows some diffuse red fluorescence of labia and clitoris. Vagina white-fluorescent, blood nonfluorescent.

FIG. 6.—R. M. (See Table IV) Vaginal swabs as seen in ordinary light.

FIG. 7.—R. M. (See Fig. 6) Vaginal swabs as seen in near ultraviolet light. Swabs from other patients showing plus 4 red fluorescence (Tables IV and V) were as intensely fluorescent as these.

FIG. 8.—A. E. Labia and clitoris of patient with putrid lochia as seen in ordinary light. Temperature had been elevated to 102° for 3 days, but normal on fifth postpartum day at time of examination.

FIG. 9.—A. E. (See Fig. 8) Labia and clitoris as seen in near ultraviolet light. Red-fluorescent material most intense in region of the clitoris and labia minora. Some red-fluorescent material on buttocks has come from vagina.

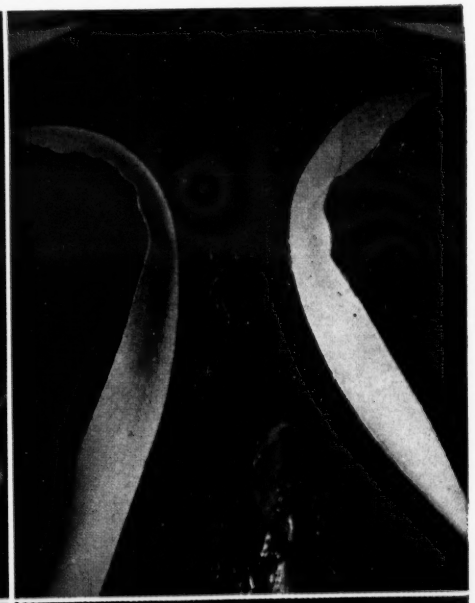
* Four color plates made possible by a grant from the Maryland Division of the Woman's Field Army of the American Society for the Control of Cancer, Inc., and a grant from the J. A. Cox Fund.

Fig. 2.

Fig. 4.

Fig. 6.

Fig. 8.



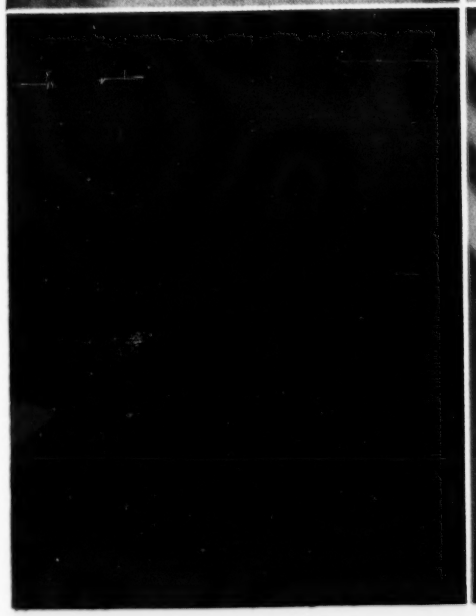


Fig. 3.

Fig. 5.

Fig. 7.

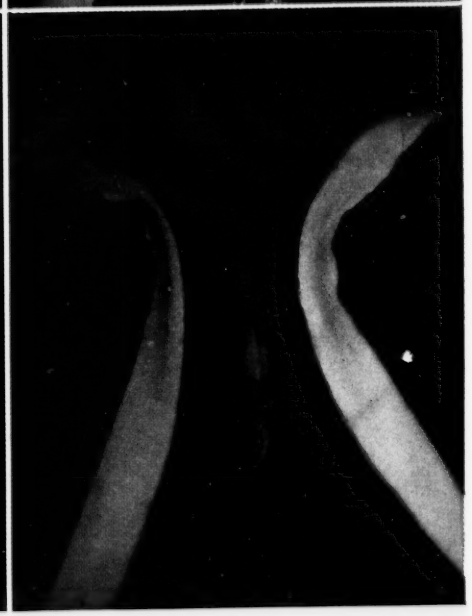


Fig. 9.

TABLE IV: NONMALIGNANT CASES SHOWING PRONOUNCED RED FLUORESCENCE

Name	Age, color	Chief complaint	General diagnosis	Cervix	Red Fluorescence: Cervix	Vagina	Clitoris	Labia minora	Labia majora	Vaginal exudates	Stage of cycle	No. of pregnancies	Remarks
R. M.	34 C	Irregular bleeding for 1 mo.	Deferred	Chronic infection, slight	+ 3	+ 2	0	+ 4	+ 4	Moderate, thick (epithel.)	Postmenstrual (2 days)	2	Conization of cervix 6 months before
"	"	(1 week later)	Deferred	"	sl.	sl.	+ 1	0	0	Slight	Intermenstrual	2	
L. H.	38 C	None	Hyperthyroidism	Chronic infection, slight	0	0	+ 4	+ 4	+ 4	Blood	Menstrual	4	Hyperthyroidism; untreated goitre 10 x 15 cm.
"	"	(11 days later)	"	"	—	—	sl.	0	0	— (not bleeding)	Intermenstrual	4	Thiouracil, high vitamins, thiamine chloride
"	"	(2 weeks later)	"	"	—	—	sl.	0	0	— (bleeding)	Menstrual (2nd day)	4	Treatment: same as above
"	"	(2 days later)	"	"	—	(orifice + 4)	+ 4	+ 4	0	— (not bleeding)	Postmenstrual (1st day)	4	Treatment: same as above
S. B.	23 C	Discharge	Draining cul-de-sac abscess	Normal	+ 4	+ 4	+ 4	+ 4	+ 4	Moderate, thick, brown, foul	Postmenstrual (1st day)	1	Laparotomy later: infected ectopic pregnancy
F. P.	28 C	Scanty menses; lack of libido	Fibromyomas	Normal	+ 3	+ 3	+ 4	+ 2	+ 1	Moderate (epithel.)	Early intermenstrual	1	1 miscarriage at 4 mo. several yrs. ago
G. B.	32 C	Short of breath	Asthma	Normal	0	(urethra and vag. orifice + 2)	0	0	0	0	Premenstrual	6	
I. S.	28 C	Pregnant	Pregnant	Normal pregnant	0	(yellow fluor. within vag. orifice + 3)	0	0	0	(epithel.)	Pregnant (4 mo.)	4	Creases of hands and nail beds, red fluor., 2

TABLE V: CARCINOMA CASES SHOWING PRONOUNCED RED FLUORESCENCE

Name	Age, color	Chief complaint	General diagnosis	Cervix	Red fluoresence: Cervix	Vagina	Clitoris	Labia minora	Labia majora	Vaginal exudates	Stage of cycle	No. of pregnancies	Remarks
H. D.	76 W	A growth	Carcinoma, clitoris	—	—	—	+ 4 (prepuce + 4)	+ 3	+ 2	—	Postmenopause	0	Lesion about 2 cm.[3]
L. R.	40 C	Discharge	Carcinoma, cervix, squam. cell (recurrent)	2 cm. area of necrosis	+ 4	0	0	0	0	Necrotic on cervix	Postmenopause (1 yr. ago radiation)	4	Parametrial involvement
M. L.	50 C	Bleeding for 1 yr.	Carcinoma, cervix, squam. cell	Enlarged, irregular, eroded, sl. bleeding	0	0	+ 3	+ 4	0	Blood	Postmenopause	4	Parametrial involvement
E. G.	45 C	Pains and bleeding, 6 mo.	Deferred	Lacerated, otherwise normal	0	0	+ 2	sl.	0	Blood	Menstrual ?	1	Radium for bleeding 1 yr. ago
"	"	(1 week later)	Deferred	"	+ 4	+ 4	+ 1	0	+ 2	Watery, foul, copious	Postmenstrual (3 days)	1	Anesthetic death 2 days after last exam., before D & C possible
"	"	(1 week later)	Carcinoma, cervix or corpus (autopsy)	"	+ 4	+ 4	+ 2	+ 2	+ 1	Watery, foul, copious	Intermenstrual	1	Autopsy: extensive Ca., cervix or corpus
M. D.	29 W	Bleeding	Carcinoma, cervix, squam. cell	Enlarged; 2 cm. area of necrosis	+ 4	0	+ 4	+ 4	+ 4	Blood in vagina	Menstrual (2nd day)	6	Bleeding and discharge since childbirth 8 mos. ago
E. L.	33 W	Spotting	Carcinoma, cervix, squam. cell	Everting cauliflower-like growth 5 cm.[3]	0	0	0	0	0	Exudate, slight on surface	Premenstrual	1	Patient took a tub bath with soap 8 hrs. before last exam.
"	"	(5 days later)	"	"	—	—	+ 2	+ 4	+ 2	Bleeding	Menstrual (4th day)	1	
"	"	(2 days later)	"	"	0	0	+ 4	0	0	Exudate, sl. on surface of lesion	Postmenstrual	1	

other uteri, which were removed because of myomas, were examined. The endometrium had only a very pale orange fluorescence such as we found in normal uteri. The cervix had been recently amputated in 2 women examined; there was slight orange fluorescence around the surgical site on 1 of these.

One woman, complaining of metrorrhagia over a number of years, was examined 2 days after her regular menstrual period. She showed intense red fluorescence of the genital tract. There was no bleeding present at the time. There were 2 women with draining cul-de-sac abscesses, of whom 1 showed intense red fluorescence of the vaginal secretions. Three weeks previously, when her cul-de-sac was opened, both pus and small clots of old blood drained profusely from the opening. Laparotomy, 1 week following the examination for fluorescence, revealed an infected ectopic pregnancy. The other patient had had a draining cul-de-sac abscess for about 1 year but the foul pus that filled her vagina was not red-fluorescent.

Four women were seen who showed definite atrophy of the genitalia. One was 91 years old; the vulva of another showed the white, silvery appearance of pronounced atrophy; 2 showed the typical picture of extreme senile vaginitis and cervicitis with petechial hemorrhages. Red fluorescence was not present on the genitalia of any of these.

The hyperthyroid cases have been mentioned previously.

Thirty-three women are included under the classification of miscellaneous. This group consisted of:

1 hemangioma of the liver
1 healed lymphogranuloma venereum involving the vagina and urethra
11 hysterectomies (3 total)
1 aplastic anemia
1 relaxation of the vagina with partial prolapsus uteri
1 diabetic vulvitis
3 acute pelvic inflammatory adnexal masses
4 retroverted uteri (1 subinvoluted)
1 severe cystitis
2 trichomonas vaginitis (with profuse vaginal discharge)
5 obesity

In none of these 31 women was red fluorescence very intense. Small amounts were found on the clitoris in 7, the labia minora in 1, and in the vagina in 1.

Fourteen women with pulmonary tuberculosis were seen. Eight had far advanced lesions and were poorly nourished. None showed intense red fluorescence. Six had minimal pulmonary tuberculosis, of whom 1 showed slight red fluorescence of the labia majora. One of the far advanced showed some red fluorescence of the labia minora. Red fluorescence of the clitoris was equally divided between the 2 groups.

Two granulomatous lesions deserve special mention. One woman had condylomata acuminata scattered over both labia. There was a condylomatous mass surrounding the rectum and extending into the vaginal orifice that measured about 6 cm. in diameter and 2 cm. in thickness. Fluorescent examination of the genitalia was not made. After removal by electrocautery the mass showed definite red fluorescence of the base and in the crypts. The other woman had extensive granuloma inguinale, with a completely nonepithelialized surface from above the region of the inguinal glands to the anus. The labia and the clitoris had been excised one year previously because she had elephantiasis of the vulva, which interfered with walking and sitting. The patient came to the clinic with the complaint of diminishing caliber of the urinary stream. The urethra could not be identified, the vagina was occluded by the granulomatous process. Under near ultraviolet light there were spots of red fluorescence scattered over the perineal and vulval regions. These were especially distinct around the periphery of the lesion.

Table VI shows the distribution of fluorescence in the lochia pads.

Most of the fluorescent material observed in the 121 women was contained in exudates about the genitalia that could be removed. This material often contained gram-positive and gram-negative organisms, necrotic material, old epithelial cells, but very few well-preserved white blood corpuscles. Some of the red-fluorescent material was within the epithelial integument of the organs studied.

Most cosmetics and soaps used by women were found to be not red-fluorescent. However, some dark brown powders, Lifebuoy soap, and antiseptic solutions like merthiolate fluoresce red in varying degrees. Some fluorescent material had a pH on the acid side, some on the alkaline side.

DISCUSSION

Red fluorescence has been found on the genitalia of about two-thirds of the women examined. It was found in large amounts in about 1 of 10. Most of these women were in the menstrual or postmenstrual stage of the cycle. Large amounts were also found in some postpartum cases. This would indicate that most of the porphyrin seen is derived from blood coming from the uterus. However, since it was present in some women and not in others there must have been either a qualitative or quantitative difference in the state of the genitalia of the different women examined. That red fluorescence is related to bacterial flora is indicated by its frequent appearance coincident with putrid lochia. The bacteriology of the lochia in 565 patients is given by Stander (17). The organisms represented are mostly ones that have been shown to be relatively nonpathogenic for common laboratory

animals. However, some of these, which are normally saprophytic, undoubtedly become pathogenic at times. Another bacterial organism not mentioned by Stander, but common on the genitalia of both women and men, is the *Mycobacterium smegmatis,* which, also, is not pathogenic either for man or laboratory animals. The tubercle bacillus, a member of the same family, is known to be a porphyrin-producing organism (10), and we have seen colonies of smegma bacillus that showed some red fluorescence.

The bacteriology of carcinoma of the cervix has been studied and summarized by Hurdon (14), who states: "Pyogenic bacteria are always present in cervical cancer and probably in most cancers of the corpus. Saprophytic organisms are also found in the foul, necrotic layer that lines so many craters where the bacterial action is entirely superficial. Hemolytic streptococci, virulent or avirulent strains, are found in some of the most advanced stages."

TABLE VI: THE INCIDENCE OF RED FLUORESCENCE ON LOCHIA PADS

Patients	POST PARTUM DAYS 1	2	3	4	5	6	7	8	9	10	11
A. W.					R 0	S 0	RS 0	A 0	S + sl.	S 0	SA + 0
G. G.						SA + 1	S + 1	S + sl.	A + sl.	A + sl.	A + 1
H.								A + sl.	A + 1	A + 0	
A. J.					S + 4		RS + 4	S + 2	S + 4	S + 2	
S.				S + 2	S + 3	RS + 1	S + 3	S + 1	A + 1	A + 2	
O. O.			RS 0	RS 0	S 0	S 0	RS 0	S 0	S + sl.	S + sl.	
McL.			S 0	S + 3	S 0	S + 1	S + sl.	S 0	SA + sl.	A + sl.	
E. H.								A + 2	A + 2		
W.						R + 2	RS + 1	RS + 1	S + 4		
T.								A 0			
A.								A + sl.			
C.								RS + 1			
W.							S + sl.	SA 0			
H.				RS + 1	RS + 3	S + 2	S + 1	S + 4			
E. S.		S 0	RS 0	S 0	S 0	S + sl.	S + sl.	A 0			
C. M.		RS 0	S 0	S + 2	S + 2	S + 1	S + 1	SA + 1			
E. S.			S 0	S + sl.	S + 1	S + 1	S + sl.	SA + 2			
E. M.		R 0	R 0	S + sl.	A 0	S 0	S + sl.				
M. M.		R + 2	R + 2	S + sl.	S + sl.	S + 2	S + sl.				
M. Mc.		R 0		R + sl.		S 0	A 0				
A. W.		R 0	RS 0		S 0	S + 1	SA + sl.				
L. S.	R 0		S + 2	S + 1	S + sl.	A + sl	A + sl.				
M.					S 0	S + 1					
E.					S + 1	S + 3					
S.		RS 0	S 0	S 0	RS 0	S + 3					
L. R.		R 0	R 0	S + sl.	S + 2						
R.			RS 0	RS 0							
R.		R 0	S 0	RS 0							
F. S.	R 0	R 0	S 0	RS 0							
S.	R 0	RS 0									
L.		RS 0									
P.		SA 0									
E. J.		S + 1									
S.	R 0										

SUMMARY

Total pads	4	14	14	16	17	18	17	17	9	7	2
No. of pads showing fluor.	0	2	2	10	9	14	14	11	9	5	1
Total degree of fluor. (sl. = 0.5)	0	3	4	11.5	17	20	15	15.5	14	5.5	1

R = rubra. S = serosa. A = alba.

Recent studies (20) show that metaplasia of the uterine epithelium is a common occurrence in mice that develop pyometra under excessive estrogen stimulation. Ewing (2), in discussing the importance of inflammatory reactions, states: "Chronic inflammation of mucous membranes precedes cancer, as in uterus, stomach, urinary passages, nares, and with cholelithiasis. It is highly probable that specific chemical irritants, some of them dependent upon the presence

of a peculiar microbic flora of these regions, is an essential factor in the production of cancer in these regions. This subject has never received any careful attention." The hypothetical "specific chemical irritant" may well be the porphyrins produced by bacterial or histolytic decomposition of blood.

The high incidence of red fluorescence on the clitoris and labia minora, and its presence on the glans penis, indicate that the red-fluorescent material may be derived from glandular sources. Glandular sebaceous secretions are abundant on all these sites. The protoporphyrin secreted by the harderian gland of rats, which is probably a modified sebaceous gland, is somewhat comparable (1, 8, 11, 19).

It is of interest and of some importance to know whether the red fluorescence seen in women corresponds in any way to the distribution of carcinoma of the region studied. The series of cases presented is not large but there are a few findings suggesting such a relationship. Carcinoma of the cervix is much more frequent in the multiparous woman than in the nulliparous. The most intense fluorescence we found in the women studied was that which occurred in association with foul lochia during the puerperium. These red-fluorescent lochia have been seen as long as 6 weeks after delivery. Hence there must be large amounts of porphyrin present all during this time. Furthermore, these secretions are pooled in the region of the cervix and fornix of the vagina. Conversely, in the condition of prolapsus uteri, which exhibits a low-cancer incidence, a similar condition would not be present. At necropsy the uterus of 1 woman with foul lochia showed intense red fluorescence extending throughout the uterine cavity. Therefore it is obvious that infected lochia are red-fluorescent all through the postpartum period. Red fluorescence was also found about the cervix in most of the untreated carcinoma patients. It is conceivable that it was present as the lesion developed. The rare lesion of primary carcinoma of the vagina occurs from 1 per cent to 3 per cent as frequently as carcinoma of the cervix (14). It is more common in multiparous women, and slightly more common in the upper half of the posterior wall of the vagina than elsewhere. This lesion would also have a chance to develop in the vagina where pooled porphyrin existed.

Carcinoma of the vulva occurs about 5 per cent as frequently as carcinoma of the cervix (18). It is primarily a disease of old age and is seen as frequently in nulliparous as in multiparous women. About half of the cases arise from the clitoris or tissues adjacent to it (18). In our series of cases the intensity of red fluorescence of the clitoris was striking. It was also rather intense in the region adjacent to the carcinoma of the clitoris previously mentioned. Red fluorescence was observed about the margins of a condylomatous and a granulomatous lesion. These lesions are known to precede carcinoma of the vulva (18).

It is of interest, too, that several cases (Tables IV and V) showed intense red fluorescence primarily about the vaginal orifice. This is the region where metastases of carcinoma of the uterus often occur. The reason for the predilection of carcinoma for this site is not known.

The control cases of males were few in number, but red fluorescence of the glans, especially around the corona, was seen. This is the most common site of carcinoma of the penis. No fluorescence was seen in patients who had been circumcised. Carcinoma of the penis is rare in circumcised men. On the other hand, in oriental men, where the prepuce is well developed and phimosis is common, the incidence of cancer of the penis is very high, 10 to 15 per cent of cancer in men (15). These observations suggest that there is a parallelism between the occurrence and distribution of red-fluorescent exudates and cancer incidence in these organs and tissues.

In conclusion, from the observations above it is apparent that the areas of the female genital tract known to be the site of a high cancer incidence are all associated intermittently or continuously with red-fluorescent secretions or exudates in many women. The incidence of the occurrence of red fluorescence in the various organs and tissues parallels the cancer incidence, with the exception of the cervix. On the cervix, which exhibits the highest cancer incidence, we observed red fluorescence less frequently than on the clitoris or the labia. However, we have seen that in the presence of foul lochia intense fluorescent material may be pooled about this site continuously for a month or more. Moreover, since the occurrence of red fluorescence has been shown to be intermittent, the actual incidence is probably higher than is indicated by the data in this report. Furthermore, the incidence of red-fluorescent material on all regions was found to be much higher than the cancer incidence.

If several factors are involved as causes of a disease, which is probably so in cancer, the frequency of the disease is not exclusively determined by the frequency of any one factor. In such conditions the incidence for any given site is controlled by and related to the coincidental occurrence of all the etiological factors. The relationships revealed by this study are compatible with the concept that porphyrins may be one of the factors in the etiology of cancer.

SUMMARY

1. The genitalia of 121 women were examined for fluorescence in near ultraviolet light.

2. The clitoris was red-fluorescent in 40 cases, the labia minora and majora were so in 16 and 13 cases respectively. Red-fluorescent secretion or exudates were observed in the vagina in 12 and on the cervix of the uterus in 11 women. When red-fluorescent material was observed on the vulva it was always most concentrated on and near the clitoris. Red-fluorescent material was also found in the region of the corona of the glans penis in uncircumcised males.

3. The genitalia of some women are intermittently red-fluorescent. Red fluorescence was observed most often during the menstrual or postmenstrual phase of the cycle.

4. Putrid lochia are intensely red-fluorescent, and the fluorescent material may persist for a month or two.

5. The occurrence of red fluorescence was not definitely related to any organic disease.

6. An attempt was made to discover the source of the red-fluorescent material. Most of it was thought to arise from the bacterial or histolytic decomposition of blood that exudes from the uterus. Some evidence points to a direct bacterial or glandular (cervical or sebaceous glands) origin of the red-fluorescent material.

7. The possibility of a relationship between cancer incidence and the occurrence of red-fluorescent secretions or exudates is discussed.

8. While the observations lend support to the hypothesis that the red-fluorescent material is one of the causative factors in the development of malignancy, no proof of this was obtained. The significance of these observations and direct proof or disproof of such an etiological relationship are left for future investigation.

REFERENCES

1. Derrien, E., and Turchini, J. Sur l'accumulation d'une porphyrine dans la glande de Harder des Rongeurs du genre *Mus* et sur son mode d'excrétion. Compt. rend. Soc. de biol., **91**:637-639. 1924.
2. Ewing, J. Neoplastic Diseases. A Treatise on Tumors. Fourth edition. Philadelphia & London: W. B. Saunders Company. 1940, pp. 101, 600.
3. Figge, F. H. J. Near-Ultraviolet Rays and Fluorescence Phenomena as Aids to Discovery and Diagnosis in Medicine. Bull. School Med. Univ. Maryland, **26**:165-176. 1942.
4. Figge, F. H. J. Fluorescence Studies on Cancer. I. Porphyrin Metabolism, Harderian Gland Fluorescence, and Susceptibility to Carcinogenic Agents. Cancer Research, **4**:465-471. 1944.
5. Figge, F. H. J., and Clarke, C. D. The Basic Technique of Kodachrome Photography of Fluorescence Phenomena. J. Lab. & Clin. Med., **27**:1606-1610. 1942.
6. Figge, F. H. J., Jones, E. G., and Wolfe, Geraldine F. Fluorescence Studies on Cancer. III. The Extraction and Identification of Porphyrins from the Red-Fluorescent Exudates on the Genitalia of Women. Cancer Research, **4**:483-486. 1944.
7. Figge, F. H. J., and Lillienfeld, A. Unpublished data.
8. Figge, F. H. J., and Salomon, K. Prevention of Porphyrin Incrustations on Pantothenic Acid-Deficient Rats by Harderian Gland Ablation. J. Lab. & Clin. Med., **27**: 1495-1501. 1942.
9. Figge, F. H. J., Strong, L. C., Strong, L. C., Jr., and Shanbrom, A. Fluorescent Porphyrins in Harderian Glands and Susceptibility to Spontaneous Mammary Carcinoma in Mice. Cancer Research, **2**:335-342. 1942.
10. Fischer, H., and Orth, H. Die Chemie des Pyrrols. Vol. II, 1st half. Leipzig: Akademische Verlagsgesellschaft m. b. H. 1937, p. 480.
11. Graffelin, A. L. Porphyrin-Secreting Harderian Gland of the Albino Rat. Anat. Rec., **79**:25. 1941.
12. Guthrie, D., and Bache, W. The Infrequency of Carcinoma of the Cervix with Complete Procidentia. Ann. Surg., **96**:796-800. 1932.
13. Hartwell, J. L. Survey of Compounds Which Have Been Tested for Carcinogenic Activity. Federal Security Agency, U. S. Public Health Service. Washington: 1941.
14. Hurdon, Elizabeth. Cancer of the Uterus. Oxford Medical Publications. London: Humphrey Milford, Oxford University Press. 1942, pp. 1, 30, 158.
15. Noble, T. P. Carcinoma of the Penis in Siam. Brit. J. Urol., **5**:242-248. 1933.
16. Shimkin, M., and Mider, G. B. Induction of Tumors in Guinea Pigs with Subcutaneously Injected Methylcholanthrene. J. Nat. Cancer Inst., **1**:707-725. 1941.
17. Stander, H. J. Williams Obstetrics. A Textbook for the Use of Students and Practitioners. 8th edition. New York, London: D. Appleton-Century Company. 1941, p. 1258.
18. Taussig, F. J. Diseases of the Vulva. Gynecological and Obstetrical Monographs. New York & London. D. Appleton & Co. 1923, pp. 113, 141, 151.
19. Thomas, M. J. Contributions a l'étude des porphyrines en biologie et en pathologie. I. Genèse du noyau tetrapyrrolique. Bull. Soc. chim. biol., **20**:471. 1938.
20. Weinstein, L., Gardner, W. U., and Allen, E. The Effect of Estrogenic Hormones on the Bacterial Content of the Uterus. Yale J. Biol. & Med., **16**:43-51. 1943.

Fluorescence Studies on Cancer*

III. The Extraction and Identification of Porphyrins from the Red-Fluorescent Exudates on the Genitalia of Women

Frank H. J. Figge, Ph.D., Edward G. Jones, M.D., and Geraldine F. Wolfe

(From the Departments of Anatomy and Gynecology, University of Maryland, School of Medicine, Baltimore 1, Maryland)

(Received for publication April 20, 1944)

The hypothetical considerations that led to the discovery of red-fluorescent material on the cervix of the uterus and other parts of the genitalia of women have been stated previously (3, 4, 8). Since the property of red fluorescence is not limited to porphyrins, an attempt has been made to extract and identify the red-fluorescent substances to ascertain whether or not the red-fluorescent material contained porphyrins, and, if so, which ones. It was found that the various types of genital exudates contained protoporphyrin 9, mesoporphyrin 9, deuteroporphyrin 9, and coproporphyrin. While this analysis indicates that the red-fluorescent material was derived from decomposed blood, none of the sources that had been postulated (8) could be excluded. The fact that protoporphyrin 9 and coproporphyrins were found was of interest because animals that exhibit a high susceptibility to spontaneous and induced cancer excrete these porphyrins throughout most of their life span (4).

METHODS AND MATERIAL

Red-fluorescent exudates were collected from the several sources in different ways. The red-fluorescent material on the cervix of the uterus and in the fornix of the vagina was removed on cotton swabs (8); see Plate I, Fig. 1, of paper II. Cotton pledgets were used to collect red-fluorescent material from the region of the clitoris and vulva. The pads used to absorb lochia were obtained from the obstetrical department.[1] The red-fluorescent ones were selected by examination in near-ultraviolet light and the red-fluorescent areas of the lochia pads were cut out and extracted. A smaller number of nonfluorescent pads were cut up and extracted in a similar manner.

At first, Fischer's acetic acid extraction as modified by Dobriner was followed (2, 5). Later, the ethyl-acetate acetic acid method as outlined by Grinstein-Watson was tried and used for most of the extractions (6). The porphyrins removed by these solvents were extracted with HCl solutions and purified by driving the porphyrins back and forth between aqueous HCl solutions and ether in the usual manner.

Finally, the various porphyrins in purified porphyrin mixtures were separated by repeated fractional separation from ether according to their different HCl numbers, each of the various porphyrin fractions so isolated was finally extracted from ether with 25 per cent HCl, and the absorption bands were determined with a spectrometer.[2] The various fractions were also tested for solubility in chloroform and 20 per cent NaOH. No attempt was made to determine the absolute amounts of the various porphyrins isolated, since the starting material could not be collected in a quantitative way. The relative amounts of the porphyrins from a given source were estimated by comparative fluorometry.

RESULTS

The red fluorescence in all the exudates examined was related to the presence of porphyrins. Protoporphyrin 9 and coproporphyrin were found in variable amounts in all the exudates extracted. In addition, mesoporphyrin or deuteroporphyrin or both were found in some of the exudates (Tables I and II).

Most of the porphyrins from cervico-vaginal exudates were extracted from ether with 0.2 per cent HCl and 0.6 per cent HCl. The absorption bands for the 0.2 per cent HCl extract (Table I) indicated that this was mainly coproporphyrin. Not enough could be obtained to determine melting points so that identification of the type of coproporphyrin was not possible. The porphyrins in the 0.2 per cent HCl fraction were soluble in 20 per cent NaOH and insoluble in chloroform. The fact that 42 per cent of the porphyrins from the cervical swabs was coproporphyrin makes it

* This work was aided by grants from The Anna Fuller Fund and The International Cancer Research Foundation.

[1] We are indebted to the Department of Obstetrics in the University Hospital for the collection of these pads.

[2] We are indebted to Dr. H. B. Wylie, of the Department of Biochemistry, for use of the spectrometer.

appear probable that some of this or all was coproporphyrin type III derived from heme or protoporphyrin 9. The absorption bands of the material extracted from ether with 0.6 per cent HCl in some cases corresponded closely to the known absorption bands of mesoporphyrin; in other cases, to those of deuteroporphyrin. In most cases the absorption bands indicated a mixture of mesoporphyrin and deuteroporphyrin. Some of both of these porphyrins would have been extracted with this concentration of HCl. Both were probably present in varying ratios in this fraction, which contained 53 per cent of the total porphyrin.

Table I: Comparison of Main Absorption Bands of the Porphyrins Isolated from Red-Fluorescent Genital Exudates with the Absorption Bands of Known Porphyrins

Source of or type of porphyrin	% HCl used to extract from ether	Main absorption bands, millimicrons, solvent 25% HCl			Solubility	Diagnosis
Cervix	0.1	591.0		548.5	Soluble in	Coproporphyrin
Cervix	0.1	591.2		549.0	20% NaOH	"
Cervix	0.2	591.0		549.0		"
Lochia	0.2	591.0		550.0		"
Coproporphyrin I	0.08	592.2		548.7*F	Soluble in	
Known values III	0.09	593.6		550.2†S	20% NaOH	
Cervix	0.6	591.0	572.0	548.4	Insoluble in	Deuteroporphyrin
Cervix	0.6	591.2		549.0	20% NaOH	Mesoporphyrin
Cervix	0.6	593.2		550.2		Mixtures
Lochia	0.6	592.8	570.0	550.4		
Lochia	0.6	592.9		550.0		
Mesoporphyrin	0.5	593.0	572.4	550.7*F	Insoluble in	
Deuteroporphyrin	0.4	591.5		548.5*F	20% NaOH	
Known values		591.0		548.0†S		
Cervix	2.0	600.8		555.0	Insoluble in	Protoporphyrin
Cervix	5.0	602.0		556.0	20% NaOH;	
Lochia	5.0	602.8		557.3	soluble in	
Lochia	5.0	602.0	582.5	557.0	chloroform	
Protoporphyrin	2	602.4	582.2	557.2*F		
Known values						

* F—Fischer's values.
† S—Schumm's values.

Only 5 per cent of the total porphyrin from the vagina and cervix was protoporphyrin. However, enough of this was obtained to determine 2 absorption bands. The wave lengths absorbed corresponded closely to the 2 main absorption bands of protoporphyrin. This material could be extracted from 5 per cent HCl solutions with chloroform.

The red-fluorescent material from lochia was fractionated according to the same plan as that outlined for the material collected on vaginal swabs. The same types of porphyrins as those recovered from cervical swabs were found, but there was a much higher percentage of protoporphyrin in lochia. The relative amount of the various porphyrins found in each type of exudate is listed in Table II.

Another porphyrin appeared to be present in lochia as well as in material from the cervix, but it could not be identified. It was most abundant in 1 per cent HCl extracts and was regarded as a decomposition product of protoporphyrin that formed when the isolation and identification procedures required a long time (2 to 4 weeks). The absorption bands in 25 per cent HCl were between those of protoporphyrin and hematoporphyrin.

The material removed from the clitoris and labial folds did not yield a sufficient amount of porphyrin for its type to be identified. The absorption bands of the initial extract were faint, but indicated that porphyrins were present. The porphyrins present in this extract showed differential solubility in different concentrations of HCl but could not be further identified.

Table II: Relative Amounts of Porphyrins Present in Red-Fluorescent Exudates

Type of porphyrin	Exudate from cervix and vagina, per cent	Lochia, per cent
Protoporphyrin 9	5	45
Mesoporphyrin and deuteroporphyrin	53	18
Coproporphyrin	42	37

DISCUSSION

Hauser (7), in 1929, had observed the red fluorescence of the external genitalia of women while routinely examining hospital patients in ultraviolet light. He did not examine the cervix of the uterus and was

unable to collect enough of the material from the external genital organs to determine the absorption bands. He found the fluorescent substances to be soluble in HCl ethyl or methyl alcohol, but insoluble in water, alcohol, chloroform, or ether. The porphyrins that we (8) ourselves collected were in most cases soluble in the latter solvents.

Hauser was able to show that the red-fluorescent material had a photosensitizing effect on paramecia, which indicated that some porphyrin might have been present. Since he observed the red-fluorescent material only after genital bleeding, he also postulated its origin from blood. After painting blood on female external genitalia that did not fluoresce red he noted the development of the red-fluorescent material in some instances. This origin of the porphyrin from the blood agrees with the well-known fact that certain bacteria have the ability to produce protoporphyrin from blood (5, 9).

While Hauser's data, as well as most of our own, indicate an origin of the porphyrin from blood by bacterial and/or histolytic degradation, other mechanisms may also be involved. The frequent occurrence of red-fluorescent material on the corona, frenulum, and prepuce of uncircumsized men was not compatible with the concept that these porphyrins were derived from extravasated blood. If the presence of porphyrins on the cervix proves to be related to the bacterial degradation of blood in this semi-anaerobic environment, then according to the hypothesis set forth the bacteria involved would play an indirect etiological role in the development of carcinoma of the cervix. By a process of degradation of the menstrual, lochial, or menorrhagic blood that occurs frequently and abundantly in the human subject, these organisms produce porphyrins that, by acting intermittently and over a long time, may hypersensitize the cervical cells to the action of estrogenic or carcinogenic substances.

The data on the occurrence and physiological action of the four porphyrins found have been abstracted (15). The following facts appear to be pertinent. Mesoporphyrin has a stronger photodynamic action on paramecia than the otherwise more toxic hematoporphyrin. It occurs in small amounts in oil shale, asphalt, paraffin oil, and wax. In these instances it usually accompanies mesoetioporphyrin and certain chlorophyl decomposition products. It has been prepared by reducing protoporphyrin and hematin. Deuteroporphyrin 9 has been found in small amounts in human feces, especially when the diet contained blood or when there was bleeding from the stomach. It is regarded as a decomposition product of blood in the gastrointestinal tract. It also forms in blood that is permitted to decompose *in vitro*.

Coproporphyrins have no photosensitizing influence. They are found in abundance in the feces as well as in the urine of patients with porphyria and after lead and sulfonal poisoning. Coproporphyrin I is normally present in the serum of many animals and in the human subject. It is increased in pernicious anemia and kidney diseases, and is frequently present, together with protoporphyrin, in human saliva, in the substantia alba on the teeth and gums, and in the openings of the sebaceous glands in certain areas. Many yeasts manufacture coproporphyrin I and it is found in asparagus, potatoes, cow's milk, hen's eggs, and many vegetable and animal cells; it has been demonstrated also in cultures of tubercle and diphtheria bacilli.

Protoporphyrin 9 has no photosensitizing action in the human subject or other mammals, but alcoholic solutions have a very potent sensitizing effect in paramecia. Protoporphyrin is found in small amounts in human feces together with coproporphyrin I, especially after the ingestion of blood-containing food, and after gastrointestinal bleeding. When air is excluded from decomposing blood or hemoglobin, protoporphyrin is formed. This occurs also in decomposing muscle and other organs such as heart, liver, spleen, lung, and uterus. The sterile autolysis of meat gives rise to large amounts of protoporphyrin. It has been produced by the inoculation of blood culture media with fecal material or bacteria from gangrenous tissues. The postulated origin of these porphyrins from blood is thus justifiable.

Mesoporphyrin is the only one of the porphyrins isolated that has a strong photosensitizing action. The potency of the various porphyrins to sensitize cells to carcinogenic stimuli may parallel the tendency to sensitize cells to light, but there is no evidence to show that this is the case. Büngeler (1) has demonstrated, however, that substances with a photosensitizing action increase the sensitivity of mice to cancer induced by light.

SUMMARY

The red-fluorescent material observed on the genitalia of women was collected, extracted, and identified. The data on the solubility and spectral absorption showed conclusively that the red fluorescence was related to the presence of porphyrins. Most of the porphyrin removed from the cervix was a mixture of mesoporphyrin, deuteroporphyrin 9, and coproporphyrin (probably type III); only 5 per cent was protoporphyrin 9. Most of the porphyrin in the lochia was found to be coproporphyrin (probably type III). Only a relatively small amount of mesoporphyrin and deuteroporphyrin was present in lochia; about half of the porphyrin in lochia was protoporphyrin 9.

The definite spectroscopic identification of protoporphyrin and other porphyrins in the exudates removed from the region of the uterine cervix in women lends support to the hypothesis that porphyrins may sensitize cells to carcinogenic stimuli.

REFERENCES

1. Büngeler, W. Über den Einfluss photosensibilisierender Substanzen auf die Enstehung von Hautgeschwülsten. Ztschr. f. Krebsforsch., **46**:130-167. 1937.
2. Dobriner, K., and Rhoads, C. P. The Porphyrins in Health and Disease. Physiol. Rev., **20**:416-468. 1940.
3. Figge, F. H. J. Fluorescence Studies on Cancer. I. Porphyrin Metabolism, Harderian Gland Fluorescence, and Susceptibility to Carcinogenic Agents. Cancer Research, **4**:465-471. 1944.
4. Figge, F. H. J., Strong, L. C., Strong, L. C., Jr., and Shanbrom, A. Fluorescent Porphyrins in Harderian Glands and Susceptibility to Spontaneous Mammary Carcinoma in Mice. Cancer Research, **2**:335-342. 1942.
5. Fischer, H., and Orth, H. Die Chemie des Pyrrols. Vol. II, 1st half, pp. 764. Leipzig: Akademische Verlagsgesellschaft m. b. H. 1937.
6. Grinstein, M., and Watson, C. J. Studies of Protoporphyrin. III. Photoelectric and Fluorophotometric Methods for the Quantitative Determination of the Protoporphyrin in Blood. J. Biol. Chem., **147**:675-684. 1943.
7. Hauser, H. Fluoreszenzerscheinungen am weiblichen Genitale. Strahlentherapie., **33**:582-587. 1929.
8. Jones, E. G., Figge, F. H. J., and Hundley, J. M., Jr. Fluorescence Studies on Cancer. II. The Red Fluorescence of the Genitalia of Women. Cancer Research, **4**: 472-482. 1944.
9. Kämmerer, H. Über Porphyrinbildung durch Darmbakterien. Klin. Wochschr., **2**:1153-1155. 1923.

The Oxidative Response of Normal and Neoplastic Tissues to Succinate and to *p*-Phenylenediamine*†

Otto Rosenthal, M.D., and David L. Drabkin, M.D.

(From the Harrison Department of Surgical Research and the Department of Physiological Chemistry, School of Medicine, University of Pennsylvania, Philadelphia 4, Pennsylvania)

(Received for Publication March 31, 1944)

Because of limitations in the morphological evaluation of growth processes, Craig, Bassett, and Salter (3) proposed an estimation of the oxidative behavior of surviving tissue slices as a functional test of malignancy. In this test the increment in oxygen consumption that results from the addition of succinate or of *p*-phenylenediamine to tissue slices in a Ringer's glucose medium is determined. The magnitude of these oxidative responses may be considered to be an index of the capacity of certain component oxidation-reduction systems, namely succinic dehydrogenase + cytochrome *c* + cytochrome oxidase, or cytochrome *c* + cytochrome oxidase or cytochrome *b*. The data reported by Craig and his associates indicated that the percentile oxidative responses of malignant neoplasms were appreciably smaller than those of their parent tissues. The greatest diminution of both absolute and percentile responses was found in chemically induced malignant tumors of liver and skeletal muscle, tissues that normally have a metabolic character very different from that of tumors in general. This is an important contribution to the concept that tumors possess a characteristic type of metabolism. However, since the striking findings were obtained only in liver and skeletal muscle, in which spontaneous neoplasms are uncommon, the evidence does not prove that the magnitude of oxidative response may be used as a general functional test in the classification of tumors. As it seemed desirable to obtain information on the extent of correlation of the oxidative behavior of tissues with their potentiality for malignancy the inquiry has been broadened to include the oxidative behavior of tissues in which spontaneous neoplasms occur with relative frequency.

In this report data will be presented upon the oxidative behavior of epithelial tissues from rabbit, rat, and man, and of spontaneous neoplasms from the two latter species. Limitation to epithelial tissues seemed advisable because there are available many more surgical specimens of carcinoma than of sarcoma. Moreover, in the resection of gastrointestinal cancers sufficient parent tissue is obtained to allow the parallel examination of normal and neoplastic specimens from the same host. Although it is obvious that in dealing with spontaneous tumors and complex parental tissues a strict comparison of homologous normal and malignant cells is not technically feasible, a survey of the magnitude of the oxidative responses in various types of normal and neoplastic tissues nevertheless should furnish information upon the range of applicability of this procedure as a functional test for malignancy.

METHODS

Preparation of the material.—Organs of freshly killed animals, or human tissues after surgical removal, were collected in ice-cooled jars. Slicing was done by means of a double-blade cutter, the tapered top and bottom plates of which extended almost to the free edges of the blades. This arrangement permitted horizontal slicing of small organs or biopsy material without changing the position of the blades owing to application of pressure. The minimal thickness of slice technically obtainable with this instrument was 0.2 mm.[1] Certain tissues, however, were either too fragile or too tough for such thin slicing. In these instances either slices of 0.3 mm. thickness,

* The expenses of this work were defrayed in part by grants from the Penn Mutual Life Insurance Company Foundation for the Study of Neoplastic Diseases and the George de Benneville Keim Fund.

† A preliminary report has appeared (7).

[1] The thickness of slice was assumed to be equal to the ratio (initial wet weight of drained slice)/(area of floated slice). The specific weight of the tissue was neglected. In a previous study by one of us (O.R.) on the intensity of succinate oxidation in rat liver slices (5) the ratio (terminal dry weight × 3.3)/(initial area) was utilized as a comparative index of the thickness of slice, where the factor 3.3 represented the average ratio (wet weight)/(dry weight) in fresh liver. The average calculated thickness of slice that provided for approximately maximal rates of succinate oxidation was 0.11 mm. This value corresponds to a calculated thickness of $0.11 \times 6.3/3.3 = 0.21$ mm. in the absolute units used in the present study, because the ratio (initial wet weight of drained slice)/(terminal dry weight of slice) is approximately 6.3 for rat liver.

or minced [2] or mashed [2] tissues were employed. Specifications as to the type of preparation used are given in the tables.

Mucous membranes were separated from the submucosa by means of blunt dissection. The membranes from the descending colon and proximal rectum will be referred to as colon mucosa. The thickness of colon mucosa was about 0.2 mm. in rat and rabbit and about 0.3 mm. in man. Since this thickness was within the range of that of the slices, the membrane was merely divided into pieces of suitable weight and directly used for manometry. Gastric mucosa of man, which was approximately 0.7 mm. thick. was pinned down on a cake of paraffin and cut into long strips of 0.4 mm. width by means of the double-blade cutter. The strips were treated as slices.

The slices were collected in ice-cooled, wet chambers. They were then floated in chilled stock Ringer's solution, trimmed, drained on linen,[3] placed on pieces of glassine paper, weighed rapidly on a torsion balance, and transferred to the ice-cooled manometer flasks by means of a stainless steel microspatula. Batches of the slices, similar in number and gross appearance to those selected for manometry, were utilized for the determination of the ratio of wet to dry weight (Ww/Wdr), for assay of protein-bound phosphorus, and for histological examination. Mashed and minced tissues were treated in an analogous manner except that the exposure to stock Ringer's solution was omitted.

Manometry.—The oxygen uptake was measured by means of the direct method of Warburg (4). The gas phase was pure oxygen, the temperature 37.5° C. The general arrangement and the composition of the media have been described previously (6). In addition to 0.4 ml. of 0.11 molar sodium phosphate buffer (pH 7.4), each experimental vessel contained one of the three following mixtures: (a) 1.6 ml. of stock Ringer's solution (SR) + 0.2 ml. of 0.9 per cent saline; (b) 1.6 ml. of SR + 0.2 ml. of 0.55 molar succinate, pH 7.4; (c) 1.4 ml. of SR + 0.4 ml. of 0.275 molar *p*-phenylenediamine, pH 7.4. The *p*-phenylenediamine solution was prepared during the filling of the manometers with oxygen (at room temperature), and placed in the side bulbs of the flasks just before the cocks of the manometers were closed. It was tipped into the main compartment 5 minutes before the first reading was taken, *i.e.*, approximately 7 minutes after the immersion of the manometers in the water bath.

Readings were taken at intervals of 5 minutes for the first 20 minutes and at intervals of 10 to 15 minutes thereafter. Only the period of linear uptake of oxygen was utilized in the computation of metabolic rates. In general the period of linearity was of the order of 30 minutes in the case of succinate oxidation and of 20 minutes with *p*-phenylenediamine oxidation. The rates are expressed in terms of Q_{O_2} values (μl O_2 consumed per mgm. initial dry weight of tissue per hour) and written as positive figures. The symbol Q_{O_2} denotes the rate of oxygen consumption in the absence of added substrates (autorespiration), while $\Delta Q^{S}_{O_2}$ and $\Delta Q^{Ph}_{O_2}$ designate the increments of rate due to the addition respectively of succinate and of *p*-phenylenediamine. The $\Delta Q^{Ph}_{O_2}$ values are corrected in the usual way for the autoxidation rate of *p*-phenylenediamine.

Protein-bound phosphorus content.—The protein-bound phosphorus of the tissues was determined by a method slightly modified (8) from that described by Berenblum, Chain, and Heatly (2). Technical details and a discussion of the advantages and limitations of the method have been presented previously (8). It may be added here that the protein-bound phosphorus content of slices that had been washed with SR was consistently higher than that of freshly minced tissue, a clear indication that inert tissue matter was lost from the floating slices. This demonstrates that the activities of fresh and rinsed tissues cannot be compared correctly upon a dry weight basis.

The results of the assays are expressed in terms of micrograms of phosphorus per mgm. dry weight of tissue. This term is designated in the tables by the letter "P." Q_{O_2} values referred to the phosphorus basis [*i.e.*, (Q_{O_2}/P) values] are listed in the tables under the heading "P-Basis." The unit of reference for the oxidative response is in these instances one microgram of phosphorus instead of one milligram of dry weight. By referring the oxidation rates to the protein-bound phosphorus content an automatic adjustment is supplied for the variable factors of inert tissue mass (such as intercellular matrix) and cellular storage substances (glycogen, etc.), and an approach made thereby to the expression of oxidation rates upon the basis of cellularity.

Microscopic examination.—Microscopic examination of embedded samples of the tissues was carried out in all the experiments with neoplasms and surgical material, as well as in those experiments with animal tissues where variations in the cellular composition of individual specimens were likely to occur. For a rough numerical estimate of such variations paraffin sections of formol-fixed samples were stained with van Gieson's

[2] Mincing was accomplished by the successive use of curved surgical scissors and microscopic scissors. Mashing (employed in the case of brain cortex) was carried out in a small weighing bottle by means of a stainless steel microspatula (crystal crusher).

[3] This procedure was kindly suggested to us by Dr. William C. Stadie.

stain and the areas of the individual tissue components estimated by measurement with a cross-line eyepiece micrometer.

Remarks on the Critical Rate of Oxygen Consumption

To insure that we were dealing with the oxidative behavior of intact cells, the tissue preparations employed for manometry were chosen with the aim of avoiding, so far as possible, mechanical damage to the cellular structure. A potential factor of error, however, with the type of tissue preparations employed, is the limitation of the rate of oxygen consumption by the rate of diffusion of the reactants (oxygen, succinate, *p*-phenylenediamine) into the tissue. Due to this limitation, if the oxidative activity of a tissue is great, underestimation of its oxidative capacity is probable. This is true even when the thickness of the slices or tissue fragments is kept as low, and the concentration of the reactants as high, as is technically possible.

Table I: The Critical Rate of Oxygen Consumption

Temperature: 37.5° C
Oxygen Tension (c_o): 1 atmosphere
d = Thickness of slice (cm.)

$$Q'_{O_2} = \frac{8c_o D\,(Ww/Wdr)}{d^2} \times 60\ *$$

d	Q'_{O_2} for Ww/Wdr		
	4.5	5.5	6.5
0.02	74	90	107
0.03	33	40	47.5
0.04	18.5	22.5	27

* Derived from Warburg's equation for the limiting thickness of slice:

$$d' = \sqrt{\frac{8c_o D}{A}}$$

where D is Krogh's diffusion constant ($1.4 - 10^{-5}$ cc. oxygen per square centimeter tissue per minute, when the gradient of oxygen tension is 1 atmosphere per centimeter); and A the rate of oxygen consumption (cc. oxygen per cc. tissue per minute).

When the rate of oxygen consumption of a tissue is at a given level, it is possible to calculate from Warburg's equation (4) the "limiting thickness of slice" at which all the tissue cells are considered to be provided with oxygen. By means of a simple algebraic transformation of this equation (Table I) a *critical rate of oxygen consumption* may be computed for any particular thickness of slice. The critical rate, as defined by the equation, is the maximum rate of cellular oxygen consumption under conditions that permit oxygen to reach every cell. It must be recognized that if the oxygen consumption rate of the cells is greater than the critical rate, calculated for the slice, anoxic cells must be present. It follows that *the oxidative capability of a tissue sample is underestimated when the measured rate of oxygen consumption is greater than the critical rate.*[4] Since thickness of slice cannot be measured closer than ±10 per cent, our *index of approach to optimal conditions is 20 per cent below the critical rate.*

The critical rate, expressed in Q_{O_2} units, will be designated Q'_{O_2}. In Table I Q'_{O_2} values are tabulated that cover the range of thicknesses of slice and of (Ww/Wdr) ratios encountered under our experimental conditions. These data are utilized in the evaluation of the experimental results—a criterion not hitherto employed.

No definite critical rate can be computed for irregular particles such as are present in minced or mashed tissues. Because of the small size of such particles, however, the critical rates may be considered similar to those calculated for cylinders or spheres of corresponding diameter. The critical rates for bodies of this shape are respectively twice and thrice the critical rate for a slice of the same diameter.

Critical rates with reference to the diffusion of succinate and of *p*-phenylenediamine cannot be calculated because the penetration rates of these substances into tissues are not known. The substrate concentration (*M*/20) employed in this study was chosen because it produced approximately maximal oxidative response in rat liver slices of 0.2 mm. thickness without considerably changing the osmotic pressure of the medium, and, in the case of *p*-phenylenediamine, without causing severe toxic effects. Besides using higher substrate concentrations than Craig and his associates (3), we omitted glucose from the medium in order to avoid the interference of side reactions with the oxidation of the two substrates under test.

EXPERIMENTAL

Table II summarizes experimental results upon the oxidative behavior of normal epithelial tissues. An inspection of the data reveals that the magnitude of the oxidative responses varied greatly with the type of tissue and, to some extent, with the species examined. In several instances the differences as brought out by our technic may have fallen short of the actual differences in the oxidative activity of these tissues, since the rate of total oxygen consumption was not always below the critical rate for the given type of preparation (see values marked with an asterisk). In

[4] It is mathematically possible to calculate the rate expected for complete oxygen diffusion through the tissue sample. This rate is equal to (square of observed rate)/(critical rate) as derives from the equations given in Table I. However, an application of a corresponding correction to our experimental data seemed not justifiable since experimental proof for the validity of the computation was technically not feasible.

regard to the tissue preparations with indefinite particle size (brain, lung) the conclusion seems warranted that the rate of succinate oxidation was well below the critical level since the oxygen consumption rate in the presence of *p*-phenylenediamine was several times higher.

Notwithstanding the possible contraction of differences among tissues with high oxidative activity, an approximate classification of normal epithelial tissues with reference to their oxidative behavior seems permissible. When the dry weight is used as a standard of reference, three types of oxidative response may be distinguished. (a) a high response to both succinate and *p*-phenylenediamine (kidney and liver), (b) a medium high response to succinate combined with a high response to *p*-phenylenediamine (brain and submaxillary gland); and (c) oxidative responses of a low order of magnitude toward both substrates (mucosa and lung). An evaluation of the results upon the basis of protein-bound phosphorus changes this grouping to a certain extent. In the succinate series brain cortex shifts to the group with the highest oxidative response, while in the *p*-phenylenediamine series a widening of the range of the oxidative responses affecting group (a) and (b) is the most prominent feature.

In Table III data are assembled on the oxidative behavior of individual benign and malignant neoplasms of rat and man. Only experiments with tumors have been included in which the area of the respective type of neoplastic tissue amounted to at least 30 per cent of the total area of the microscopic section. Even with such a selection the oxidative activity of the nonneoplastic tissue components must be taken into account before the oxidative behavior of the specific tumor cells can be evaluated.

Experiments with human scirrhous mammary cancers, which contained 85 to 95 per cent of stroma, indicated that connective tissue exhibits a low oxidative activity per unit mass of dry tissue. Upon a protein-bound phosphorus basis, however, the oxidative activity of these scirrhous tumors was close to that of the cellular cancers listed in the table (Nos. 1, 4, 5, 6, 7, 9). Hence acellular connective tissue in the amounts recorded in Table III will probably neither distort nor mask the oxidative behavior of the neoplastic cells if the protein-bound phosphorus is used as a standard of comparison. On the other hand, the stroma of the gastrointestinal cancers was more cellular than that of the scirrhous tumors because of the presence of various kinds of migratory cells, though the mass of the latter appeared to be small in comparison with that of the carcinomatous regions. The oxidative ac-

TABLE II: THE OXIDATIVE RESPONSE OF NORMAL EPITHELIAL TISSUES TO SUCCINATE OR *p*-PHENYLENEDIAMINE

(Mean values ± standard error)

Tissue	Species	No. of exps.	Ww/Wdr	Wdr—Basis *			P—Basis †		
				Q_{O_2}	$\Delta Q^{S}_{O_2}$	$\Delta Q^{Ph}_{O_2}$	Q_{O_2}	$\Delta Q^{S}_{O_2}$	$\Delta Q^{Ph}_{O_2}$
KIDNEY CORTEX	Rat	5	4.40	11.4	54.7 ± 3.94 ‡	63.0 ± 3.16 ‡	4.2	20.5 ± 1.26	24.0 ± 1.88
(Slices: 0.2 mm.)	Rabbit	3	4.59	6.4	29.8 ± 1.89	53.9 ± 3.92 ‡	2.0	9.6 ± 0.79	16.9 ± 1.74
	Man	2	5.22	5.9	31.2 ± 0.10	27.3 ± 3.25	1.7	9.5 ± 1.45	8.0 ± 0.30
LIVER	Rat	9 §	4.65	1.4	46.7 ± 1.51	38.3 ± 2.36	0.3	11.6 ± 0.60	9.6 ± 0.63
(Slices: 0.2 mm.)	Rabbit	3	5.14	0.7	40.1 ± 6.61	37.7 ± 6.56	0.2	10.8 ± 0.97	10.2 ± 1.42
BRAIN CORTEX	Rat	3	4.54	3.7	13.3 ± 0.87	85.4 ± 0.74	2.3	8.5 ± 0.70	52.8 ± 2.10
(Mash)	Rabbit	3	5.35	4.0	11.6 ± 0.04	68.4 ± 2.92	3.2	9.3 ± 0.21	54.4 ± 2.39
SUBMAXILLARY GLAND	Rat	4	4.22	6.1	15.2 ± 2.66	45.5 ± 0.56 ‡	1.0	2.5 ± 0.56	7.4 ± 0.59
(Slices: 0.3 mm.)	Rabbit	3	5.24	2.0	20.0 ± 1.24	40.8 ± 2.15 ‡	0.3	2.9 ± 0.17	5.8 ± 0.26
COLON MUCOSA	Rat	5	5.70	4.8	3.5 ± 1.11	14.8 ± 2.80	0.7	0.5 ± 0.17	2.1 ± 0.44
	Rabbit	3	6.76	9.2	4.2 ± 0.15	19.3 ± 0.99	1.5	0.7 ± 0.04	3.1 ± 0.26
	Man	9	6.21	5.1	5.9 ± 0.28	16.1 ± 1.13	0.8	1.0 ± 0.07	2.7 ± 0.31
STOMACH MUCOSA	Man	5	6.80	5.0	5.6 ± 0.97	10.3 ± 1.03	0.9	1.0 ± 0.17	1.8 ± 0.21
LUNG	Rat	3	4.70	1.3	1.7 ± 0.18	8.0 ± 1.32	0.4	0.5 ± 0.07	2.2 ± 0.25
(Mince)	Rabbit	3	4.40	1.9	3.2 ± 0.29	9.9 ± 1.48	0.4	0.7 ± 0.09	2.2 ± 0.42
	Man	1	5.84	1.8	1.9	3.9	0.7	0.8	1.6

* Calculated per mgm. dry weight.

† Calculated per mgm. protein-bound phosphorus.

‡ In these experiments the rate of total oxygen consumption (*i.e.*, Q_{O_2} + respective ΔQ_{O_2}) was identical with or not more than 20 per cent less than the corresponding "critical rate" as given in Table I.

§ In three of these experiments the response to only succinate was tested.

tivity of the migratory cells,[5] though uncertain, seems to be similar to that of cancer cells, while their protein-bound phosphorus content[5] may be even higher. Finally, the oxidative activity of smooth muscle, also present in these tumors, was found to be slightly above that of the cellular cancers, while the protein-bound phosphorus content was extremely low (P=1.4). Thus upon a protein-bound phosphorus basis the effects of smooth muscle and migratory cells should partly cancel out.

Examination of the data in Table III discloses that upon a protein-bound phosphorus basis the oxidative responses of neoplasms were of a fairly uniform order of magnitude. The variations observed appear to be a function of the behavior of the nonneoplastic tumor components rather than of the morphological character or the degree of malignancy of the neoplastic cells. It will be noticed, furthermore, that the oxidative activity of this group of tumors was strikingly lower than that of normal kidney, liver, and brain; less significantly different from that of submaxillary gland; and virtually identical with the activities of normal mucosa and lung.

It should be emphasized that in every experiment with gastrointestinal carcinomas the adjacent normal mucosa was tested simultaneously. A presentation of the individual data appeared superfluous, since upon neither a phosphorus nor a weight basis were distinct differences revealed between the cancers and parent tissues.[6]

TABLE III: THE OXIDATIVE RESPONSE OF NEOPLASMS TO SUCCINATE OR *p*-PHENYLENEDIAMINE

Exp. No.	Site or designation of tumor †	Type of tumor	Histology: Percentage area ‡ Neoplastic	Stroma	Smooth muscle	Manometry *: $\frac{Ww}{Wdr}$	Wdr—Basis §: Q_{O_2}	$\Delta Q^{S}_{O_2}$	$\Delta Q^{Ph}_{O_2}$	P ‖	P—Basis ¶: Q_{O_2}	$\Delta Q^{S}_{O_2}$	$\Delta Q^{Ph}_{O_2}$
						RAT							
1	Walker 256 **	Carcinosarcoma	85	15	0	6.7	3.7	9.6	12.3	8.4	0.4	1.1	1.5
2	Breast ††	Adenoma	56	44	0	4.5	1.5	2.7	5.3	3.4	0.4	0.8	1.6
3	No. 70 ††	Adenoma	34	66	0	6.2	2.2	6.0	9.0	3.9	0.6	1.5	2.3
4	Neck (anterior)	Carcinoma simplex	84	16	0	5.5	4.3	6.8	13.1	9.9	0.4	0.7	1.3
5	No. 132	Myxosarcoma	100	0	0	11.5	3.7	11.1	18.3	8.0	0.5	1.4	2.3
						MAN							
6	Renal pelvis	Papillary carcinoma	95	5	0	6.7	3.3	4.1	13.3	5.6	0.6	0.7	2.4
7	Esophagus	Anaplastic carcinoma	90	10	0	6.5	1.8	5.1	10.2	5.2	0.3	1.0	2.0
8	Stomach	Colloid carcinoma	30	17	0	11.8	1.2	1.9	8.2	2.8	0.4	0.7	2.9
9	Stomach	Adenocarcinoma	77	23	0	4.8	2.2	4.3	11.3	6.9	0.3	0.6	1.6
10	Sigmoid	Adenocarcinoma (mature)	54	43	0	6.5	2.2	5.5	9.3	4.7	0.5	1.2	2.0
11	Rectum	Adenocarcinoma	54	33	5	6.8	2.4	4.2	17.4	4.3	0.6	1.0	4.0
12	Rectum	Adenocarcinoma	58	35	7	5.7	1.0	12.4	19.0	5.2	0.2	2.4	3.7
13	Rectum	Adenocarcinoma (polypous)	32	42	10	6.1	0.5	3.8	11.5	5.2	0.1	0.7	2.2
14	Rectum	Adenocarcinoma	34	66	0	7.4	1.1	7.9	9.0	5.7	0.2	1.4	1.6

* Three types of preparation were employed: (a) mince (Experiments 6, 8, 13); (b) 0.2 mm. slices (Experiment 1); (c) 0.3 mm. slices (all other experiments). In the experiments with slices the total rates of oxygen consumption were always below the corresponding critical rates.

† The three transplantable tumors used in this study (Walker 256, No. 70, and No. 132) were derived from spontaneous tumors of the mamma. Tumor No. 70 is sometimes referred to as a benign adenocarcinoma.

‡ The following constituents were found in addition to those listed in the table: 53 per cent colloid matter (Experiment 8); 3 per cent and 8 per cent mucosa (Experiments 10 and 11 respectively); 14 per cent mucosa and 2 per cent lymphatic tissue (Experiment 13).

§ Calculated per mgm. dry weight.

‖ Microgram protein-bound phosphorus per mgm. dry weight.

¶ Calculated per mgm. protein-bound phosphorus.

** This specimen was examined 2 weeks after transplantation. Older (more necrotic) tumors exhibited weaker oxidative responses, especially toward succinate. The results of these experiments were omitted from the table as their representativeness seemed questionable; compare Albaum and Potter (1).

†† The animals from which these tumors were obtained had produced litters shortly before. The tumors contained milk cysts. The microscopic pictures resembled those of normally developed mammary glands.

[5] This is suggested by data on the oxidative activity of leukocytes and lymphocytes (3), and on the protein-bound phosphorus content of lymph nodes (8).

[6] While mucous membranes are not purely epithelial tissues their composition appears to be quite similar to that of the adenocarcinomas studied by us with reference to the relative areas of epithelial and nonepithelial components. The following data were obtained by us for gastric mucosa and colon mucosa respectively: epithelium (glandular and surface) 56 per cent, 32 per cent; propria 25 per cent, 62 per cent; smooth muscle 11 per cent, 6 per cent; lymphatic tissue 8 per cent, 0 per cent.

Craig, Bassett, and Salter (3) employed the percentile oxidative responses as a criterion of the oxidative behavior of tissues, a procedure that would make unnecessary the protein-bound phosphorus correction of the quantitative data. Under our experimental conditions the range of the percentile oxidative increments to succinate, computed from the data in Tables II and III, was as follows: 3,000 to 6,000 in liver, 450 to 550 in kidney, 250 to 350 in brain and submaxillary gland, 50 to 150 in mucosa and lung, and 120 to 300 in neoplasms, if extremely high values (Table III, Nos. 12 and 14) are omitted. Although the grouping of the normal tissues thus brought out was fairly similar to that obtained with our previous method of evaluation, only the percentile oxidative responses of liver tissue were far above the range of the responses of tumors.

A still greater alteration in the grouping of the normal tissues and more overlapping between normal range and tumor range was brought about by comparing the percentile increments to *p*-phenylendiamine with the absolute responses to this substrate. The following series of percentile oxidative responses to *p*-phenylenediamine demonstrates this point: 2,500 to 5,500 (liver), 1,500 to 2,500 (brain), 450 to 850 (submaxillary gland and kidney), 200 to 600 (lung), 200 to 300 (mucosa), and 300 to 800 for tumors (with the omission of the extremely high values in Experiments 12 and 13 of Table III).

DISCUSSION

In comparing the experimental results reported here with those obtained by Salter and his group (3, 9) it should be emphasized that our experimental arrangement deviates from that of the earlier investigators in regard to three technical details: (a) the use of higher concentrations of succinate or *p*-phenylenediamine; (b) the omission of glucose as a basal substrate; and (c) the use of thinner slices than are customary, or even appropriate, in the usual type of metabolic studies.

Our technic, which had previously proved to provide an adequate estimate of the succinic oxidase activity of liver slices (5), facilitates the saturation of the cellular enzyme systems with the substrates to be tested. It also achieves retardation of the spontaneous tissue respiration through the washing out of cellular substrates and soluble catalysts from the extra thin slices without impairing the activity of the succinic dehydrogenase or the cytochrome system. However, the degree of retardation is not constant for different tissues. It is greatest in liver and smooth muscle, and less pronounced in kidney cortex.

The varying extent of retardation of autorespiration and nonspecific oxidative activity of the tissues introduces some uncertainty into the evaluation of oxidative responses to specific substrates. For example, the validity of corrections applied for autorespiration may be questioned. The oxidation of the test substances may not be superimposed upon the autorespiration, since the saturation of the tissues with external substrates may possibly suppress completely the utilization of cellular material. Fortunately, our experimental results are not materially altered whether the increment in the rate or the total rate in presence of the test substances serves as the standard of comparison. Secondly, the use of increment in oxygen consumption upon addition of succinate as a measure of succinic dehydrogenase activity presupposes that the increased oxygen uptake serves exclusively for the oxidation of succinate to fumarate, and no farther. While this apparently holds for thin liver slices, it has not been demonstrated to apply in the other tissues studied. Oxidation of succinate beyond fumarate may possibly occur.

While the factors discussed above concern the correct position of the base line for judgment of oxidative increments there exists, in addition, the ceiling effect that the penetration rate of the reactants exerts upon the rate of oxygen uptake. This effect depends upon the relationship between oxidative activity and critical respiratory rate for the given type of tissue preparation. The technical limitations that prevent exclusive usage of optimal preparations have been dealt with in the beginning of this paper.

It is thus obvious that data on the enzymatic activity of incompletely destroyed tissues can serve merely as an approximation of their true enzymatic power, and that only pronounced differences between tissues should be considered significant. Nevertheless, the magnitudes of the absolute oxidative increments to succinate or *p*-phenylenediamine that have been observed by us in kidney, liver, brain, lung, and the Walker carcinosarcoma of the rat, correspond roughly to the distribution of succinic dehydrogenase and cytochrome oxidase as found by Schneider and Potter (10) with specific methods of assay. It appears reasonable, therefore, to assume that the experimental technic and the method of evaluation here employed furnish a fair index of the activity of the enzymatic system under comparison.

By expressing their results in terms of the ratio of increment in oxidative response over basal respiration Craig and his colleagues (3) employ a standard of comparison that is independent of variations in the cellularity of the tissues. The addition of glucose by these workers to the media aims at producing a basal respiration of uniform quality, regardless of variations in the concentration of cellular substrates. It is our opinion that the latter purpose can hardly be achieved

because the response to glucose of different tissues is just as variable as is the intensity of their autorespiration. Hence, stability of baseline cannot be assured by the device employed by the authors just mentioned, and variations in the denominator of their ratio may greatly influence the quantity of the percentile oxidative responses, without reflecting the activity of the succinic dehydrogenase or the cytochrome system. It is furthermore reasonable that the magnitude of the numerator of the ratio—the absolute increment in response—is technically limited by factors already discussed in connection with the critical rate of oxygen consumption. Since slices of the usual thickness and comparatively low substrate concentrations were employed in the method of Craig and his associates, the critical rate of oxygen consumption must necessarily have been lower than that provided with our experimental technic.[7]

It is therefore not surprising that among tissues with high oxidative activity only those (liver, skeletal and cardiac muscle) with comparatively low basal respiration were found to yield high percentile responses (3, 11), while kidney cortex, extremely rich in succinic dehydrogenase and cytochrome oxidase but possessing a high basal respiration, showed responses not far above the tumor level (11). On the other hand, the range of percentile responses obtained with lung (9), which is known to be poor in the enzymatic systems under discussion, overlapped both the tumor range and the range characteristic of oxidatively active tissues. These examples should suffice to demonstrate that the order of magnitude of the percentile oxidative responses appears to be not as reliable a measure of the "over-all activity," or "effectiveness," of the succinoxidase or cytochrome system as Craig and his colleagues (3) assume. Their test may, however, be indicative of other metabolic peculiarities of tissues. We question the usefulness of the test as a complement of the morphological classification of growth processes because of the narrow margin between "normal range" and "tumor range," and the unavoidable distortion of small differences through imperfections in present technic.

From our own results it appears that not only neoplasms but also gastrointestinal mucosa and lung exhibit weak oxidative responses to succinate or *p*-phenylenediamine. Other normal tissues with low oxidative response are probably myeloid tissue (11), lymphatic tissue, connective tissue, skin, and mammary gland.[8] Hence there remains only a relatively small group of tissues in which the oxidative activity surpasses that of tumors to such an extent that a measurable change of the oxidative behavior can be expected to accompany a neoplastic transformation. This group includes kidney cortex, liver, brain, and presumably smooth, skeletal (3), and cardiac (11) muscle. In all these tissues the divergence from tumor behavior is brought out most strikingly when the data are compared upon a protein-bound phosphorus basis. In the case of submaxillary gland, on the contrary, a pronounced difference is manifest only upon a mass basis. The classification of this type of epithelium with reference to its oxidative behavior remains uncertain.

Within the limited range of applicability outlined above the measurement of the oxidative activity may provide a valuable, objective criterion of the degree of dedifferentiation of neoplasms. Since in this group of tissues the intensity of the response to succinate on a protein-bound phosphorus basis furnishes the necessary information, the use of *p*-phenylenediamine, an unphysiological and rather toxic substrate, is not required. The simplicity of the technic, the small amount of material needed, and utilizability of types of preparation that preserve the tissue architecture, lend themselves for the comparison of biochemical properties with histological findings.

Although it is somewhat disappointing that the differentiation of normal from malignant forms by metabolic means appears to fail in the very types of tissue where the majority of spontaneous neoplasms arise, the existence of a correlation between level of oxidative capability and neoplastic tendency appears, nevertheless, to possess interpretative significance. This point is beyond the scope of the present study, but some consequences will be discussed in a forthcoming paper in connection with the cytochrome *c* content of tissues (8).

SUMMARY

The absolute oxidative responses to the addition of *M*/20 succinate or *p*-phenylenediamine have been studied in normal tissues of rabbit, rat, and man, and in neoplasms of the latter two species.

Thin slices or fine minces of fresh tissues were examined by means of the Warburg technic. A criterion, *the critical rate of oxygen consumption,* has been introduced to serve in the objective evaluation of "maximal" oxygen consumption rates that can be measured with the types of preparations employed.

[7] This is demonstrated by the fact that in oxidatively active tissues such as rat liver and human kidney the Q_{O_2} values in the presence of succinate or *p*-phenylenediamine as reported by Salter's group (9) are 30 to 50 per cent below the corresponding values found by us, even though our data are based upon the initial dry weight of the slices, which is 20 to 30 per cent higher than the terminal dry weight used by the other investigators.

[8] This is suggested by the results of exploratory experiments with human skin (Padgett graft) and with mammary gland from pregnant rats, and is supported by the data on the benign adenomata of the mammary gland listed in Table III. A more extensive study of these types of tissue appeared fruitless, as they are unsuitable for either thin slicing or fine mincing.

The manometric studies were accompanied by histological examination of the material.

To correct for the variable factors of inert tissue mass and storage substance the protein-bound phosphorus content of the tissues was used as a standard of reference for the quantitative data.

The following results were obtained:

1. Normal epithelial tissues fall into two main groups: (a) tissues with high oxidative responses towards succinate or *p*-phenylenediamine (kidney cortex, liver, brain cortex, and probably smooth and striated muscle); (b) tissues with low responses toward the two test substrates (gastrointestinal mucosa, lung, and possibly, skin, mammary gland, and lymphatic tissue). The position of the submaxillary gland is intermediate between the two main groups.

2. Benign and malignant rat tumors as well as human cancers show remarkably uniform oxidative responses of a low order of magnitude, similar to that found in normal group (b).

It follows that changes of the oxidative behavior incident to a malignant transformation can be expected to occur only in types of tissue belonging to normal group (a). Within this group, the divergence of the oxidative behavior from that of the parent tissue should serve as an objective criterion of the degree of dedifferentiation of neoplasms. With the method of evaluation here suggested the examination of the response to succinate alone would fulfill this purpose.

It is pointed out that the method of Craig, Bassett, and Salter (3), who utilize the percentile oxidative responses to succinate ($M/50$) or *p*-phenylenediamine ($M/100$) in a glucose medium as a standard of comparison, cannot be considered a reliable index of the activities of the succinic dehydrogenase or of the cytochrome system.

Sincere thanks are due to Dr. Shields Warren for supplying the original Walker carcinosarcoma and to Dr. Margaret R. Lewis for providing all the other tumor-bearing rats used in this study.

REFERENCES

1. Albaum, H. G., and Potter, V. R. Biocatalysts in Cancer Tissue. II. Inhibition of the Succinoxidase System by Tumor Extracts. Cancer Research, **3**:303-308. 1943.
2. Berenblum, I., Chain, E., and Heatley, N. G. The Study of Metabolic Activities of Small Amounts of Surviving Tissues. Biochem. J. **33**:68-74. 1939.
3. Craig, F. N., Bassett, A. M., and Salter, W. T. Artificial Benignancy of Neoplasm. VI. Observations on the Oxidative Behavior of Tumors, Artificially Benign Tumors, and Homologous Normal Tissues. Cancer Research, **1**: 869-879. 1941.
4. Dixon, M. Manometric Methods as Applied to the Measurement of Cell Respiration and Other Processes. Cambridge University Press. 1934, p. 45.
5. Rosenthal, O. The Intensity of Succinate Oxidation in Surviving Liver Tissue. Biochem. J., **31**:1710-1718. 1937.
6. Rosenthal, O., Bowie, M. A., and Wagoner, G. Studies in the Metabolism of Articular Cartilage. I. Respiration and Glycolysis of Cartilage in Relation to Its Age. J. Cell. and Comp. Physiol., **17**:221-233. 1941.
7. Rosenthal, O., and Drabkin, D. L. Cytochrome C Content, Nucleic Acid Phosphorus, and Oxidation Rate of Epithelial Tissues and Neoplasms. Proc. of The Physiological Society of Philadelphia, Session of October 20, 1942. Am. J. M. Sc., **204**:914-915. 1942.
8. Rosenthal, O., and Drabkin, D. L. Cytochrome *c* Content of Normal and Neoplastic Mammalian Epithelium, and Its Correlation with Body Mass. J. Biol. Chem., **150**:131-141. 1943.
9. Roskelley, R. C., Mayer, Nelicia, Horwitt, B. N., and Salter, W. T. Studies in Cancer. VII. Enzyme Deficiency in Human and Experimental Cancer. J. Clin. Investigation, **22**:743-751. 1943.
10. Schneider, W. C., and Potter, V. R. Biocatalysts in Cancer Tissue. III. Succinic Dehydrogenase and Cytochrome Oxidase. Cancer Research, **3**:353-357. 1943.
11. Warren, C. O. Tissue Metabolism Studies on Bone Marrow. Consideration in Relation to Tumor Metabolism. Cancer Research, **3**:621-625. 1943.

The Action of Heptanal Sodium Bisulfite Methylsalicylate and of 2,4,6-Trimethylpyridine on Tissue Cultures of Human and Mouse Carcinoma and Rat Lymphosarcoma*

Gladys Cameron, C. J. Kensler, and Robert Chambers

(From the Department of Biology, Washington Square College of Arts and Science, New York University, New York 3, N. Y.; and Memorial Hospital, New York 21, N. Y.)

(Received for publication April 19, 1944)

Our previous papers (3, 9) dealt with the effect of chemicals on tissue cultures of tumors of the rat liver induced by feeding *p*-dimethylaminoazobenzene. The finding that certain split products of the compound are more toxic to the normal than to the malignant tissue could be explained not by a specific difference between the malignancy or normality of the cell but by the fact that the tumors consisted largely of ductal epithelium. This epithelium was found to be more resistant than the parenchymal epithelium, irrespective of whether the tissue was of neoplastic or of normal origin. On the other hand, in the experiments on the action of tetramethyl-*o*-phenylenediamine on human and rat lymphosarcoma (5), indications of a selective toxicity were found in that the lymphoid cells of the malignant tissues were much more susceptible than lymphocytes of normal nodes.

This paper deals with the effects of heptanal [1] sodium bisulfite methylsalicylate and of 2,4,6-trimethylpyridine [2] on tissue cultures of human and mouse spontaneous mammary carcinoma, of rat lymphosarcoma, and of their normal prototypes. The suggestion of testing the compounds on tissue cultures was made by Dr. C. P. Rhoads, of Memorial Hospital. Under his direction, the second author had found that the heptanal preparation and the trimethylpyridine, as judged by the Warburg technic, inhibited the respiration of malignant more than that of normal tissue slices. We are indebted to Dr. Rhoads for the majority of the human tissues used and to Dr. J. B. Murphy, of The Rockefeller Institute, for the rat lymphosarcomas (12).

PROCEDURE

The tissues were removed under aseptic conditions and appropriate portions for culturing dissected out and cut into pieces of about 1 cu. mm. The double coverslip and Carrel flask technics were employed, the normal culture medium consisting of fowl plasma, 1 part; chick embryo extract, 1 part; and homologous serum, 3 parts.

Cultures of the human mammary gland carcinoma showed good epithelial outgrowths in about 4 to 9 days of culturing (1). The material was from metastases to lymph nodes, so that lymphocytes were present in the cultures. Cultures of the normal mammary gland tended to grow more slowly and less luxuriantly than the neoplastic tissue. For this reason, comparable outgrowths for the experimental studies were selected irrespective of the actual age of the cultures. The outgrowing epithelium of the normal glands was indistinguishable from that of the malignant in morphology and in the occasional formation of acinar nodules with fatlike droplets within the cells.

The spontaneous carcinomas of the mouse mammary gland (CFI and CFW) grew readily. These and cultures of normal mouse mammary gland, having comparable outgrowths, were used after 2 to 10 days' incubation. Cultures of lymphosarcoma and of normal lymph nodes of the rat formed luxuriant outgrowths within 24 to 48 hours. As has been recently described (5), the outgrowing lymphocytes of the normal nodes were mostly of the small type, while those of the neoplasm varied considerably in size and shape.

* This investigation was aided by The International Cancer Research Foundation, Philadelphia, and by Memorial Hospital, New York.

[1] Heptanal is known also as enanthaldehyde, enanthal, heptylaldehyde, enanthole, and *n*-heptaldehyde. The name "heptanal" is approved by the International Union of Chemistry. The heptanal sodium bisulfite addition compound, consisting of the two components in equimolar amounts, appears to be crystalline and is water-soluble. Heptanal, on the other hand, is a colorless liquid and only slightly water-soluble. The heptanal sodium bisulfite used in these experiments contained approximately 5 per cent of methylsalicylate. Kensler, in his respiration studies, found no significant difference between the heptanal bisulfite containing the salicylate and pure heptanal bisulfite.

[2] 2,4,6-Trimethylpyridine is also known as γ-collidine and 2,4,6-collidine. It is a colorless liquid, with a molecular weight of 121.18.

Cultures of embryonic chick kidney (2, 6) also were used in order to test the possible action of the heptanal preparation on a secreting organ.

The experimental solutions were added to the cultures in three ways. When Carrel flasks were employed the chemicals were added to the supernatant fluid medium. The majority of the cultures were prepared on coverslips. In some of the coverslip cultures the chemical was contained in fresh medium added to the growing cultures. In others the cultures were immersed for 1 or 2 hours in Tyrode solution containing the chemical. Control cultures were immersed in Tyrode solution alone. Soluble constituents of the immersion fluid rapidly diffuse into the clotted medium and their removal should be as readily effected by washing the cultures in Tyrode (4). The cells, being firmly imbedded in the fibrin clot, remain undisturbed during these procedures. After removal from the bathing media, the cultures were given a fresh drop of normal culture medium to supply the necessary nutriment lost in the Tyrode baths. The incubation of the cultures was then continued.

The three methods permitted considerable variation in the manner of treating the cultures with the experimental solutions. They all gave essentially the same results.

In general, the outgrowths from the explants were affected earlier than the tissue within the explants. The outgrowths are more flattened out and, therefore, are more directly exposed to the experimental medium. They also tend to be actively proliferating and contain many newly formed cells. The explants are more compact and represent the surviving residue of the original tissue.

The lethality of a compound for a particular type of cell was determined by the cessation of growth and eventual disintegration of the cells both in the outgrowth and in the explant.

EXPERIMENTAL

1. Experiments with Heptanal Sodium Bisulfite Methylsalicylate

This preparation, obtained in crystalline form, was dissolved in Tyrode solution to provide a concentration of 0.01 *M*. A fresh solution was prepared for each experiment. The concentrations were calculated on the basis of pure heptanal sodium bisulfite.

a. *Human mammary gland (325 malignant cultures from 20 cases and 62 normal cultures from 5 cases).*—Cultures of malignant and of normal human mammary gland were exposed to the heptanal preparation in concentrations ranging from 0.001 to 0.007 *M*. A toxic action on the epithelium of human mammary carcinoma was found following an exposure of 24 hours to a concentration of 0.002 *M*. After the washing and return to normal medium the epithelium underwent a slow, progressive, and complete disintegration within 4 to 5 days. On the other hand, exposure to concentration levels as high as 0.007 *M* exerted no permanent toxic effect on the epithelium of normal human mammary gland or on the fibrocytes or wandering cells in the cultures of either normal or malignant tissues. Figs. 1 and 2 are from photographs of human mammary carcinoma before and after treatment with 0.002 *M* heptanal preparation.

The toxic level for the normal tissue was not determined, owing to the difficulty of securing normal mammary gland material. However, the data described below show a clear-cut selectivity between the neoplastic and normal cells. For the neoplastic epithelium, the critical concentration of the heptanal preparation is 0.001 *M* and the lethal concentration 0.002 *M*, while the fibroblasts of the neoplastic tissue and the entire cultures of the normal gland survive in concentrations as high as 0.007 *M*.

Details of the experiments were as follows:

Malignant.—In a concentration of 0.001 *M* no observable effect was produced up to 72 hours, but later the epithelial sheets gave signs of breaking up and the cells exhibited undue granulation.

A concentration of 0.002 *M* was eventually lethal to the epithelium. The toxic effect became noticeable during the latter part of a 24-hour exposure, when the cells along the margins of the sheets and acinar nodules were destroyed. The first sign of this was the appearance of distinct cell outlines, followed by shrinkage and separation of the cells. Simultaneously, the cells became increasingly granular and finally cytolyzed. The cultures were then washed and returned to a normal medium, in which the disintegration of the epithelium continued until, after 4 to 5 days, all the epithelium, both in the outgrowth and in the explant, was completely destroyed. The fibrocytes, macrophages, and lymphocytes reacted to the 24-hour exposure only by a partial withdrawal of their pseudopodia and a slight increase in granulation of their cytoplasm, but they recovered completely on return of the cultures to the normal medium.

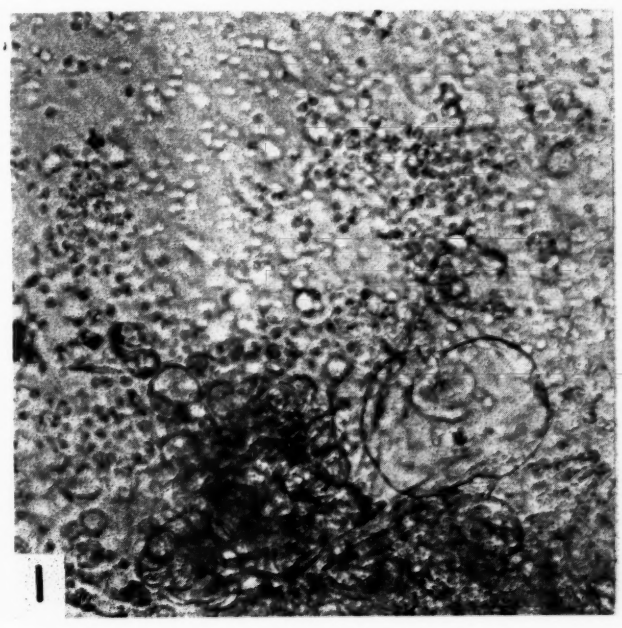
In some experiments, after washing and a return for 24 hours to the normal medium, a second dose of the heptanal preparation was given. The repeated dose hastened the destruction of the epithelium, which became complete during the following 24 hours. On the other hand, the fibrocytes and wandering cells in the cultures again recovered entirely and developed numerous mitoses.

In one series of experiments the medium containing the heptanal preparation was not changed for 4 days. In these cultures the cells of the carcinomatous epi-

thelium had completely disintegrated, while the other cell types appeared normal.

In another series of experiments with Carrel flasks a concentration of 0.007 *M* was used in the supernatant fluid, and a similar selective effect was noted. During the first 24 hours the entire culture appeared slightly abnormal. The cultures were then given alternating exposures of normal medium and a medium containing 0.004 *M* heptanal preparation at intervals of 24 and 48 hours. All the epithelium progressively degenerated and disappeared, while the other cells in the cultures recovered completely from their initial reaction and gave no further sign of being affected by the subsequent exposures.

Cultures of malignant and of normal gland showing active epithelial outgrowths were exposed to the heptanal preparation in several concentrations. A concentration of 0.01 *M* destroyed all the cultures, normal and malignant. A concentration of 0.004 *M* or repeated doses of 0.002 *M* destroyed the malignant epithelium, while a similar treatment had no effect on the epithelium of the normal gland. However, the macrophages in all the cultures gave signs of being affected. The results were not so consistent as those obtained with human mammary material but, in general, gave indications that the critical concentration for the neoplastic epithelium is lower than that for the normal.



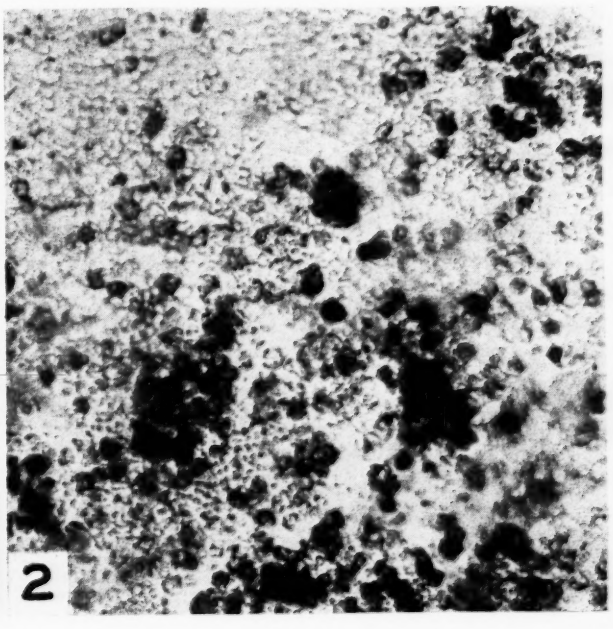


FIG. 1.—Five-day culture, human mammary carcinoma (metastasis to lymph node). Note clump of epithelial acini in lower center of photograph. Wall, with cuboidal cells, of one of the acini surrounds an irregular narrow lumen. To upper right of this acinus lies a distended, thin-walled, acinar cyst filled with fluid. Scattered throughout the field are small round lymphocytes and an occasional fibrocyte. (Living culture.)

FIG. 2.—Similar culture, after 24 hours' exposure to 0.002 *M* heptanal preparation, showing disintegration of epithelium and survival of lymphocytes. The acini have disappeared. The dark masses are clumps of cytolyzed epithelium. (Living culture.)

Normal.—Cultures of normal human mammary gland containing epithelial outgrowths comparable with those of the carcinomatous cultures were similarly exposed to a 0.007 *M* heptanal preparation followed, 48 hours later, by a dose of 0.004 *M*. Initially the epithelium showed a slight retraction of the outgrowing sheets. The fibrocytes reacted by a slight temporary granulation of their cytoplasm. The epithelial sheets soon spread out again, increased in extent, and showed growth of ducts containing fluid even after the cultures had been exposed to a second dose of the heptanal preparation, at 0.004 *M*.

b. *Mouse mammary gland (63 malignant cultures from 9 cases and 50 normal cultures from 8 cases).*—Details of the experiments were as follows:

Malignant.—In one series of experiments an exposure to 0.004 *M*, over a period of 72 hours, destroyed the carcinomatous epithelium and the macrophages were also destroyed. Three repeated doses of 0.002 *M*, at intervals of 48 hours, had the same effect, while a single dose of 0.002 *M*, following repeated doses of 0.001 *M*, showed a tendency to break the sheets into their constituent cells. When washed and returned to normal medium coherent sheets of viable cells were re-formed.

In another series of experiments an exposure to 0.004 *M* for 24 hours damaged some of the epithelial sheets. The cultures were then washed and returned to a normal medium, in which all the sheets progres-

sively disintegrated and their cells cytolyzed. Most of the wandering cells also were dead. In a few of the cultures new outgrowths of epithelium appeared and continued to develop normally, indicating that the epithelium within the explant had not been affected.

Normal.—The cultures of mouse mammary gland produced good growths of epithelium, fibrocytes, and macrophages, none of which showed any toxic reaction to 0.004 *M* except for a few of the macrophages.

c. *Rat lymphosarcoma and normal lymph nodes (245 malignant cultures from 10 cases and 105 normal cultures from 8 cases).*—The cultures were exposed to the heptanal preparation in concentrations of 0.0002 to 0.01 *M*. The action was found to be highly selective and rapid. A concentration of 0.001 *M* induced a complete destruction of the lymphoid cells of the neoplasm within 24 hours. On the other hand, all the cells in cultures of normal rat lymph nodes survived an exposure of 48 hours to a concentration of 0.0075 *M* and, in most cases, 0.01 *M*. Figs. 3 and 4 are from photographs before and after this treatment. The destruction of the neoplastic lymphoid cells was rapid, in contrast to the slow, progressive deterioration observed with the epithelium of the human carcinoma.

Details of these experiments were as follows:

Malignant.—A concentration of 0.0002 *M* in 24 hours destroyed all the lymphoid cells except a few deep in the explant. From these a fresh outgrowth sometimes occurred when the cultures were returned to a normal medium. The macrophages were unaffected at 0.005 *M* and only very slightly affected at 0.01 *M*, while the fibrocytes appeared to be actually stimulated to new growth by an exposure to 0.005 *M* and not adversely affected by 0.01 *M*.

Normal.—No toxic effect was noted on cultures of normal rat lymph nodes exposed to concentrations of 0.0075 *M* for 48 hours (Fig. 5), and only rarely was a slight toxicity observed with 0.01 *M* over a period of 72 hours. The macrophages and the fibrocytes in the cultures gave no evidence of being injured.

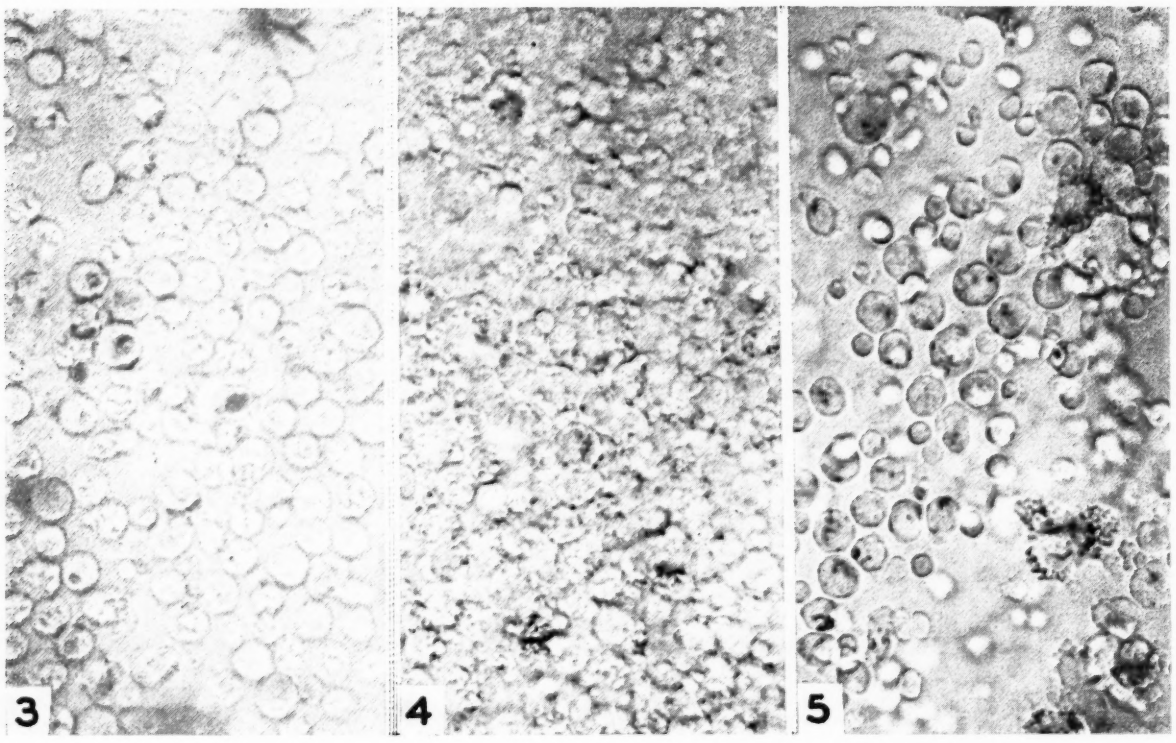


Fig. 3.—Living culture of rat lymphosarcoma.
Fig. 4.—Similar culture after 24 hours' exposure to 0.001 *M* heptanal preparation.
Fig. 5.—Living culture of normal rat lymph node exposed to 0.0075 *M* heptanal preparation. No effect.

d. *Human reticular cell sarcoma and melanoma (22 and 18 cultures respectively).*—Cultures of each of these tumors were exposed to the heptanal preparation at a concentration of 0.002 *M* for 48 hours. In the sarcoma cultures the reticulocytes were partially inactivated, while the lymphocytes showed no toxic reaction. In the melanoma cultures no destructive action was noted on any of the cells, the only difference between the treated and control cultures being a very slight shrinkage of some cells in the former. The cultures of both the sarcoma and the melanoma were transferred to a normal medium after the 48-hour treatment, and exhibited normal growth over a period of 7 days.

e. *Kidney (24 cultures).*—Cultures of chick mesonephros with secreting tubular segments were exposed to the heptanal preparation in concentrations of 0.001 to 0.01 *M*. Concentrations of 0.005 *M*, which were found from the previous experiments to be lethal for all the neoplastic cells tested, had no toxic effect on the secretory activity of these tubules during an exposure of 48 hours. In a concentration of 0.01 *M* the tubular cells were visibly damaged and secretion was inhibited.

2. Experiments with 2,4,6-Trimethylpyridine

The 2,4,6-trimethylpyridine, diluted with Tyrode solution, was tested only on mammary gland tissue.

a. *Human Mammary Gland (70 malignant cultures from 14 cases and 28 normal cultures from 4 cases).*—Cultures of malignant and normal human mammary gland were exposed to the trimethylpyridine in concentrations of 0.0025 to 0.05 *M*. The effect was similar to that of the heptanal preparation, but less consistent.

Details of the experiments were as follows:

Malignant.—As a rule, exposure of the carcinoma cultures to 0.0025 and 0.005 *M* for 24 hours produced a toxic effect on the outgrowing sheets and on the acinar nodules beyond, but not within, the explant. The fibrocytes remained normal, but the macrophages and some of the lymphocytes appeared less active than in the controls. At 48 hours the cells of the outgrowing epithelial sheets were destroyed, with indications of beginning of disintegration of the epithelium at the borders of the explants. No new growth occurred. Some of the lymphocytes were dead, but frequently a halo of recently emerged lymphocytes was observed around the explant. In a few cases the entire culture showed no toxic effect.

In three series of experiments with 0.05 *M* a specific destructive effect was obtained after the cultures were returned, 24 hours later, to a normal medium. During the initial exposure all the cells in the cultures were abnormal and very granular. The return to the normal medium resulted in a gradual improvement of the macrophages and fibrocytes while the epithelium, both in the outgrowth and in the explant, became progressively worse and, at the end of the fifth day, had cytolyzed. However, the lymphocytes were also destroyed.

In a fourth series of experiments a concentration of 0.05 *M* produced no toxic effect during 48 hours of exposure. At 96 hours the outgrowing epithelial sheets showed signs of deterioration, while all other cells in the culture, including the lymphocytes and the epithelium within the explants, appeared normal.

Normal.—Cultures of normal human mammary gland exposed to a concentration of 0.0025 *M* over a period of 72 hours consistently showed no toxic effect. An exposure to 0.05 *M* for 24 hours produced only a slight toxic effect, followed by complete recovery. When these cultures were returned to a normal medium this was made evident by a continued active growth of fresh epithelium showing ducts and acini.

b. *Mouse mammary gland (35 malignant cultures from 6 cases and 20 normal cultures from 4 cases).*—Cultures of spontaneous carcinoma and of normal mammary gland were exposed to 2,4,6-trimethylpyridine in concentrations of 0.0025 *M* to 0.05 *M*. The results were less consistent than those on the human material but nevertheless gave indications that the neoplastic is more susceptible than the normal material.

Details of the experiments were as follows:

Malignant.—Concentrations of 0.005 to 0.01 *M* produced an initial slightly toxic effect, as shown by an incipient breaking up of the epithelial sheets and a partial separation of their cells. In a concentration of 0.005 *M* the cells of the sheets underwent cytolysis only after successive applications of the 2,4,6-trimethylpyridine every 24 hours. This procedure did not affect the majority of the macrophages, or the epithelium within the explants. Evidence of this was seen in the fresh growth of epithelium that occurred from the explant when the cultures were returned to normal medium. Exposure to 0.05 *M* for 48 hours had a considerable toxic effect both on the epithelium and on the wandering cells. The epithelial sheets retracted and their cells separated, while the macrophages became rounded and granular. Return to normal medium after 48 hours had a beneficial effect both on the macrophages, which became more active, and on the epithelium, which showed evidence of new growth, though the new cells were highly vacuolated.

Normal.—The normal mammary gland epithelium was not affected by concentrations up to and including 0.05 *M*. After two successive treatments with 0.05 *M* a slight temporary retraction of the sheets was noted in one series, but complete recovery took place without transference of the cultures to normal medium.

Discussion

The results presented in this paper bear out the unpublished findings of Kensler, referred to in the introduction, concerning the existence of a physiological difference in response between certain malignant tissues and their normal prototypes. The tissue culture findings go further in demonstrating that there is a differential resistance to toxic compounds, not only between the neoplastic cell and its normal prototype but also between neoplastic cells and the other cells of the same culture.

Kensler's findings were based on the immersion of minced tissue and of tissue slices of different types of cells. Although one might suspect an inactivation of

certain respiratory enzymes, the effect may be in the nature of a general toxic action. The tissue culture experiments relate the effect to a particular type of cell. This finding may become significant when a sufficient number of compounds have been tested and a relationship found among them that might point to an attack on particular chemical systems in the malignant cell. The differential between the malignant and the normal cell might then be discovered to lie in such systems.

In the experiments described in this paper a given concentration of a compound was defined as lethal when sooner or later it caused the death of all the neoplastic cells in the outgrowth and in the explant, but of none of the other cell types in the culture. Such a dosage could be obtained with the heptanal preparation, but not with trimethylpyridine. With trimethylpyridine the maximum threshold concentration that permitted survival of the nonneoplastic cells of the culture killed the neoplastic cells already in the outgrowth, but did not prevent fresh growth from the explant.

The existence of a differential in the action of the heptanal preparation was more clear-cut in the case of the human than of the mouse mammary gland epithelium. The malignant cells of the mouse were not only more resistant than the human neoplasm but the results of different experiments with the mouse material showed greater variability than those of the human tissue.

A peculiar reaction, which may be of significance, was that the macrophages, abundant in the cultures of mouse carcinoma, succumbed to concentrations that destroyed the epithelium. This was in strong contrast to the relatively greater resistance of the lymphocytes and fibrocytes, which were abundant in cultures of the human mammary carcinoma. The suggestion is offered that the macrophages were so highly susceptible because they ingested breakdown products of the disintegrating carcinomatous epithelium. On the other hand, in the rat lymphosarcoma experiments the macrophages did not appear to be affected, and the fibrocytes were actually stimulated to further growth.

The specific destructive action of the heptanal preparation on the lymphoid elements of the rat lymphosarcoma appears to be similar to that already reported (3) for a completely unrelated compound, N,N,N′,N′-tetramethyl-*o*-phenylenediamine.

Concerning the action of aldehydes in general, Willmer and Wallersteiner (15) examined the tissue cultures of chick periosteal fibroblasts and cells of a chicken sarcoma (DBA), but found no indication that the inhibitory action of these compounds differed in the two types of cells. A similar negative result was reported by Ludford, who used heptylaldehyde (heptanal) on mixed cultures of fowl tumor cells and chick fibroblasts (10). These findings are similar to the negative results presented in this paper regarding human reticular cell sarcoma and melanoma, and suggest a comparatively greater resistance of the fibrocytic type of cell. Ludford used heptylaldehyde, which Strong (13) states is less effective than the mixture of heptylaldehyde and methylsalicylate used by us.

Garai (7) has criticized Strong's conclusions regarding the action of heptylaldehyde on mice bearing spontaneous mammary tumors, by suggesting that the toxic effect is primarily on the liver and the kidney. This was later countered by Strong (14). Our experiments show that, at least for chick kidney cultures, the secretory mechanism of the proximal tubules is not affected by the heptanal preparation in concentrations that destroy the epithelium of the mouse carcinomatous tissue.

An interesting feature regarding the action of heptylaldehyde on tumors was their extensive liquefaction. Garai and Strong both stated that the liquid consisted of a suspension of viable cells. Strong ascertained this by obtaining tumors in mice inoculated with the suspension.

The fact that treatment with heptylaldehyde causes spontaneous mouse tumors to break up into viable neoplastic cells was used to indicate that the destructive action of the chemical is, at least initially, on the fibroblastic tissue. In our experiments, however, the initial effect was a breaking up of the actual epithelial sheets, indicating a dissipation of the intercellular cement. The fibrocytes in the cultures were not affected, and in some cases were actually stimulated to further growth. The breakdown of the epithelial sheets in the cultures of human material was simultaneous with the shrinkage and destruction of the cells. In the mouse material the cells separated, but could be made to cohere again when the cultures were washed and normal medium added. Such an action would account for Strong's findings.

Another feature to be stressed in our experimental results is the latency of the toxic effect of the concentrations used. In some cases both normal and malignant cells underwent an initial reaction such as a partial retraction of the cells and the development of granulation. The normal cells gradually recovered, while the malignant cells progressively deteriorated. In other cases there was no evidence of any reaction until after 24 or even 48 hours, when the lethal effect on the malignant cells became manifest whether or not the cultures had been returned to a normal medium.

This raises the question whether the tissue culture method used here can be compared with that introduced by MacFadyen and Murphy (11). Kline and his co-workers (8) tested the effect of over 40 com-

pounds with MacFadyen and Murphy's method. They used the Flexner-Jobling rat carcinoma by mincing the tumor, half of which was immersed for 45 minutes in a solution of a given compound. The exposed and nonexposed material was then inoculated in the two opposite groins of the same rat. They introduced the precautionary measure of rendering the immersion solutions isotonic with red blood cells by adding 0.8 per cent NaCl. There is no way of telling by this method whether the neoplastic alone or both neoplastic and normal tissues have been affected.

The decided advantage of tissue cultures is that the different types of cells can be clearly distinguished in the living state. The cells can be kept under continuous observation and their reaction studied throughout the experimental procedures.

SUMMARY

1. *Action of heptanal sodium bisulfite methylsalicylate.*—a. Human mammary carcinoma cultures exposed for 24 hours to a concentration of 0.002 *M*, followed by a return to normal medium, underwent a slow, progressive, and complete disintegration of the epithelium in 4 to 5 days. In contrast to this the epithelium of normal human mammary gland cultures, and the fibrocytes and lymphocytes in cultures of either normal or malignant tissues, were not affected by prolonged exposure to concentrations up to and including 0.007 *M*.

b. Mouse mammary carcinoma cultures showed similar effects at somewhat higher concentrations (0.004 *M* or repeated doses of 0.002 *M*). Macrophages present in the malignant cultures were also damaged.

c. The action on rat lymphosarcoma was highly selective and rapid. A concentration of 0.001 *M* in 24 hours induced a complete destruction of the lymphoid cells only, while all the cell types in cultures of normal rat lymph nodes survived an exposure of 48 hours to a concentration of 0.0075 and, in most cases, 0.01 *M*.

2. *Action of 2,4,6-trimethylpyridine.*—a. In cultures of human mammary carcinoma the concentrations required to destroy all the epithelium exerted a toxic action on cultures of normal mammary gland also. An indication of selectivity lay in the outgrowing sheets, which in the carcinoma cultures were destroyed, but in the normal cultures were not, by 0.025 *M*. Lymphocytes in the cultures of normal and malignant tissues were destroyed.

b. In cultures of mouse mammary carcinoma the epithelium of outgrowing sheets was destroyed by a concentration of 0.05 *M*, which had no effect on the normal mammary epithelium. The macrophages of the malignant cultures were also destroyed.

REFERENCES

1. Cameron, Gladys, and Chambers, R. Neoplasm Studies. III. Organization of Cells of Human Tumors in Tissue Culture. Am. J. Cancer, **30**:115-138. 1937.
2. Cameron, G., and Chambers, R. Direct Evidence of Function in Kidney of an Early Human Fetus. Am. J. Physiol., **123**:482-485. 1938.
3. Cameron, Gladys, Kopac, M. J., and Chambers, R. Neoplasm Studies. IX. The Effects in Tissue Culture of N,N-Dimethyl-*p*-Phenylenediamine on Rat Liver Tumors Induced by *p*-Dimethylaminoazobenzene. Cancer Research, **3**:281-289. 1943.
4. Chambers, R., and Cameron, G. The Effect of *l*-Ascorbic Acid on Epithelial Sheets in Tissue Culture. Am. J. Physiol., **139**:21-25. 1943.
5. Chambers, R., Cameron, Gladys, and Kopac, M. J. Neoplasm Studies. XI. The Effects in Tissue Cultures of N,N,N′,N′-Tetramethyl-*o*-Phenylenediamine and Other Compounds on Malignant Lymph Nodes. Cancer Research, **3**:293-295. 1943.
6. Chambers, R., and Kempton, R. T. Indications of Function of the Chick Mesonephros in Tissue Culture with Phenol Red. J. Cell. & Comp. Physiol., **3**:131-167. 1933.
7. Garai, F. Heptyl Aldehyde-Sodium Bisulfite: Toxicity and Effect on Spontaneous Mammary Carcinoma in Mice. Cancer Research, **1**:144-145. 1941.
8. Kline, B. E., Wasley, W. L., and Rusch, H. P. Tumor Inhibitor Studies. I. The Effect of Pure Chemical Compounds on Tumor Takes. Cancer Research, **2**:645-648. 1942.
9. Kopac, M. J., Cameron, Gladys, and Chambers, R. Neoplasm Studies. X. The Effects in Tissue Culture of Some Split Products of *p*-Dimethylaminoazobenzene on Rat Liver Tumors. Cancer Research, **3**:290-292. 1943.
10. Ludford, R. J. Cited in Report of the Director. Imperial Cancer Research Fund, 36th Annual Report. 1937-1938, pp. 11-12.
11. MacFadyen, D. A., and Murphy, J. B. A Method for the Study of Induced Interference with Transplantable Tissue Growth. J. Exper. Med., **70**:461-473. 1939.
12. Murphy, J. B., and Sturm, E. The Transmission of an Induced Lymphatic Leukemia and Lymphosarcoma in the Rat. Cancer Research, **1**:379-383. 1941.
13. Strong, L. C. Synergistic effect of Heptyl Aldehyde and Methyl Salicylate on Spontaneous Tumors of the Mammary Gland in Mice. Yale J. Biol. & Med., **11**:207-218. 1939.
14. Strong, L. C. The Effect of Heptyl Aldehyde-Sodium Bisulfite on Spontaneous Tumors of the Mammary Gland in Mice. Cancer Research, **1**:473-476. 1941.
15. Willmer, E. N., and Wallersteiner, K. The Effects of Certain Aldehydes upon the Growth of Cells in Vitro. J. Physiol., **96**:16P. 1939.

Retarding Effect of Glyceraldehyde on Benzpyrene Sarcoma Formation in Mice*

J. F. Riley, M.B., F.R.C.S.Ed., and F. Pettigrew

(From the Wilkie Surgical Research Laboratory, University of Edinburgh, Edinburgh, Scotland)

(Received for publication March 23, 1944)

In an earlier experiment Riley and Wallace (8) showed that 0.5 cc. *M*/10 propionaldehyde injected subcutaneously into mice twice weekly for the 16 weeks during which tumors from a previous injection of 0.5 mgm. benzpyrene were to be expected, reduced the tumor yield from 36 to 22 per cent.

In tissue culture it has been demonstrated that the growth and cellular activity of chick periosteal fibroblasts and of the cells of a dibenzanthracene chicken sarcoma are both inhibited at similar concentrations by a large series of aldehydes (11). Before such a generalization for aldehyde inhibition *in vivo* can be accepted, it is necessary to test the effect on tumor emergence of glyceraldehyde, which differs from other aldehydes in three ways. First, glyceraldehyde is itself a normal intermediate of phosphorylating carbohydrate breakdown. Second, glyceraldehyde in crystals and fresh solution exists in dimeric (dimolecular) form and hence closely resembles glucose. Like glucose, dimeric glyceraldehyde has a similar action in inhibiting the initial step of phosphorylating glycolysis (1). Third, after being in solution for over 3 days glyceraldehyde is entirely converted into its monomeric (monomolecular) form, and as such is believed to be a specific inhibitor of direct sugar breakdown without phosphorylation. This last effect, the inhibition of glycolysis, is brought about by only one optical component, *l*-glyceraldehyde, of the racemic mixture (2, 5).

The inhibition by glyceraldehyde of anaerobic and aerobic glycolysis in tumor tissue was first pointed out by Mendel (4), and subsequently reported for chick embryo by Needham and his associates (6, 7). The question whether a special form of carbohydrate breakdown other than phosphorylating glycolysis is actually involved in the inhibition of glycolysis by glyceraldehyde has been raised by Macfarlane (3), although she was unable to offer a complete explanation of the specific inhibition of the *l*-isomeride. Süllmann (9, 10) has suggested that the inhibitory action of glyceraldehyde on carbohydrate breakdown may be due chiefly to fixation of triose phosphate by aldol condensation whereby compounds are formed that take no further part in the glycolysis.

In view of these remarks it was felt desirable to repeat our previous experiment, using glyceraldehyde in place of propionaldehyde.

EXPERIMENT

Two hundred immature male mice, 4 to 6 weeks old, of an inbred (Medical Research Council) strain were used in the experiment. These were divided into four equal groups: A, B, C, and D.

All four groups received in the right flank a single subcutaneous injection of 0.7 mgm. benzpyrene dissolved in 0.1 cc. sterile olive oil. Since the subsequent injection of glyceraldehyde was likely to bring about a reduction in the total number of tumors, a larger dose of benzpyrene was chosen than in the previous experiment in order to raise the tumor yield.

From the 11th to 27th weeks the animals in Groups A, B, and C received further subcutaneous injections twice weekly. Group A received 0.5 cc. *M*/10 "fresh" glyceraldehyde in Ringer-Locke solution; Group B received 0.5 cc. "old" glyceraldehyde in Ringer-Locke solution; and Group C received 0.5 cc. 0.9 per cent *d*-glucose in Ringer-Locke solution. Group D remained as a control. The *M*/10 solutions of glyceraldehyde were prepared by dissolving 0.9 gm. solid glyceraldehyde (Schering-Kahlbaum product) in 100 cc. of solvent and the fresh glyceraldehyde and *d*-glucose solutions were injected within an hour of preparation. The old glyceraldehyde solution was allowed to stand from 3 to 7 days before being injected. The glucose control was added in order to assess a possible pseudoglucose inhibitory effect of the fresh (dimeric) glyceraldehyde, and *d*-glucose was chosen since it is only in this form that glucose is utilized in tissue metabolism. It is from *d*-glucose that the normally occurring *l*-lactic acid (sarcolactic acid) is derived. All the animals were examined twice weekly for the appearance of tumors and the experiment was carried on for 40 weeks.

* Because of the difficulties of international communication the authors have not read proof of this article.

RESULT

As had been anticipated, tumors began to appear from the 11th week onwards in mice that had received an initial injection of benzpyrene. Autopsy was performed on any animal that died; mice bearing large tumors were killed and the tumors were examined histologically. Neither by loss in weight nor in general appearance did the mice in Groups A, B, or C show signs of toxicity as compared with those of the control Group D. The number of deaths in non-tumor-bearing mice in all groups was small and not more than 8 per cent of the animals in any group died before the period of aldehyde or glucose injections was completed.

The control Group D (benzpyrene only) yielded a total of 86 per cent of tumors, and of these 58 per cent had appeared by the 19th week. Group C (benzpyrene and *d*-glucose) yielded 78 per cent of tumors, of which 60 per cent had appeared by the 19th week. In Groups A and B (benzpyrene and aldehyde) the appearance of tumors was retarded, and although Group A (benzpyrene and fresh glyceraldehyde) gave a final yield of 72 per cent of tumors only 38 per cent had appeared by the 19th week. Group B (benzpyrene and old glyceraldehyde) showed the lowest tumor yield. This was 68 per cent, and of these only 36 per cent had appeared by the 19th week. No tumor in any group was seen to regress throughout the course of the experiment. The results are set out graphically and in the form of a table.

DISCUSSION

From the graph and table it will be seen that while the repeated injection of old glyceraldehyde produced the greatest degree of inhibition of benzpyrene sarcoma formation in mice, the curves of tumor emergence for both the fresh (dimeric) and old (monomeric) solutions of glyceraldehyde are so similar that no significant difference in the actions of the two solutions can be deduced. The repeated injection of *d*-glucose, on the other hand, gave rise to an initial acceleration in the appearance of tumors although the final yield was 8 per cent less than the number of tumors in the control group, which received benzpyrene only. Thus the action of both fresh and old solutions of glyceraldehyde resembles that of propionaldehyde in retarding the appearance of benzpyrene sar-

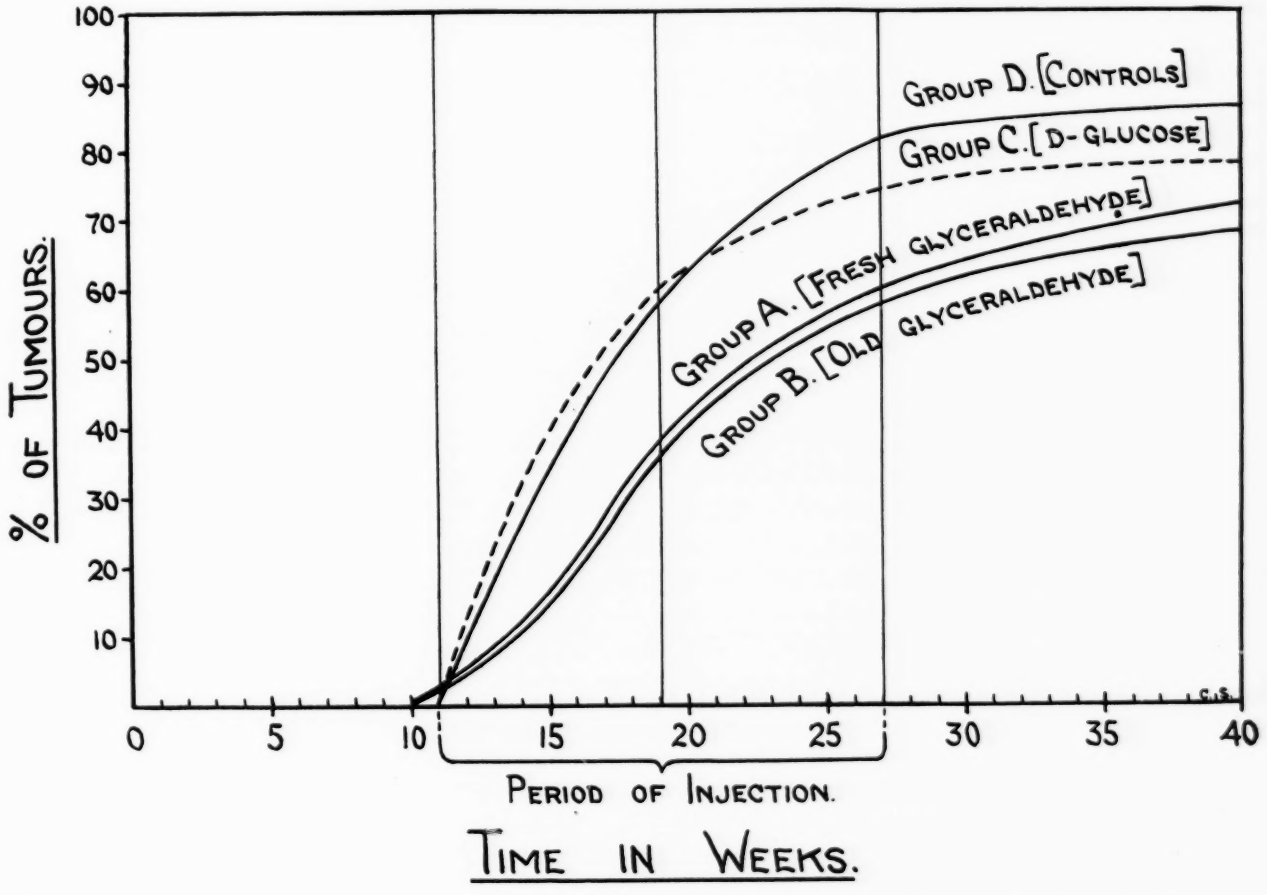


FIG. 1.—Delayed appearance of benzpyrene sarcoma, and slight reduction in tumor yield, after glyceraldehyde injections.

TABLE I

	Tumors before 11th week	Tumors from 11th to 19th weeks	Tumors from 19th to 27th weeks	Tumors from 27th to 40th weeks	Died without tumor	Survived: no tumor	Total percentage of tumors
GROUP A: Benzpyrene and fresh glyceraldehyde	1	18	11	6	5	9	72
GROUP B: Benzpyrene and old glyceraldehyde	1	17	11	5	7	9	68
GROUP C: Benzpyrene and *d*-glucose	0	30	7	2	2	9	78
GROUP D: Benzpyrene only	0	29	12	2	1	6	86
		period of injection					

comas in mice. It remains to be determined whether differences of this magnitude are significant.

SUMMARY

Under the conditions of the experiment, 0.5 cc. *M*/10 glyceraldehyde injected subcutaneously into mice twice weekly for the 16 weeks during which tumors from a previous injection of 0.7 mgm. benzpyrene were to be expected, resulted in a delay in the appearance of tumors and a slight reduction in the tumor yield. This effect was essentially similar whether "fresh" (dimeric) or "old" (monomeric) glyceraldehyde was used and resembles the inhibition, previously recorded in a comparable experiment, in which propionaldehyde was employed. It remains to be determined whether differences of this magnitude are significant.

We wish to record our gratitude to the British Empire Cancer Campaign for a gift of benzpyrene and to thank Professor J. R. Learmonth, of Edinburgh; Dr. H. Lehmann, of Cambridge; and Dr. B. Mendel, of Toronto, for their interest and advice.

REFERENCES

1. LEHMANN, H. Glycogen Breakdown in Muscle Extract and Yeast Juice. Nature, London, **141**:470-471. 1938.
2. LEHMANN, H., and NEEDHAM, J. The Action of Glyceraldehyde on Carbohydrate Breakdown. Enzymologia, **5**: 95-99. 1938.
3. MACFARLANE, MARJORIE G. The Phosphorylation of Carbohydrate in Living Cells. Biochem. J., **33**:565-578. 1939.
4. MENDEL, B. Krebszelle und Glycerinaldehyd. Klin. Wchnschr., **8**:169-170. 1929.
5. MENDEL, B., STRELITZ, FRIDA, and MUNDELL, DOROTHY. l-Glyceric Aldehyde and Tumor Metabolism. Science, **88**:149-150. 1938.
6. NEEDHAM, J., and LEHMANN, H. Intermediary Carbohydrate Metabolism in Embryonic Life. VIII. Glyceraldehyde and Glucolysis. Biochem. J., **31**:1913-1925. 1937.
7. NEEDHAM, J., and NOWINSKI, W. W. Intermediary Carbohydrate Metabolism in Embryonic Life. Biochem. J., **31**: 1165-1184. 1937.
8. RILEY, J. F., and WALLACE, A. B. Stimulation and Inhibition of Connective Tissue in Mice Following Injection of Benzpyrene. Brit. J. Exper. Path., **22**:24-28. 1941.
9. SÜLLMANN, H. Über die Hemmung der Glykolyse durch Glycerinaldehyd. Biochem. Ztschr., **296**:325-347. 1938.
10. SÜLLMANN, H. Die Hemmung der Milchsäurebildung im Muskelextrakt durch Glycerinaldehyd. Enzymologia, **5**: 372-382. 1939.
11. WILLMER, E. N., and WALLERSTEINER, K. The Effects of Certain Aldehydes upon the Growth of Cells "in vitro." Proc. Physiol. Soc., **96**:16-17. 1939.

Multiple Primary Tumors in Dogs

R. M. Mulligan, M.D.

(From the Department of Pathology, University of Colorado, School of Medicine, Denver 7, Colorado)

(Received for publication April 13, 1944)

From the point of view of comparative oncology the subject of multiple tumors has always been of great interest, particularly with regard to human neoplasms. Since a high incidence of spontaneous tumors is found in dogs, the cases of multiple canine tumors recorded in the literature have been surveyed in order to determine the age, sex, and breed of the dogs afflicted and the types and sites of the neoplasms encountered.

Any discussion of multiple tumors in dogs may be properly prefaced by an analysis of the papers by Goodpasture and Wislocki (8), by Goodpasture (7), by Cohrs (3), and by Pallaske (14) since they were the only thoroughgoing investigations of all the organs of old dogs at routine necropsy found in the literature. In the first paper were described 15 old dogs, which were included with 35 other old dogs discussed in the second paper. The 50 dogs studied were 37 males and 13 females, which were chosen because of such evidences of old age as decay and loss of teeth, grayness of hair, and presence of senile cataract. The average age range was about 9 to 14 years. Projecting above the surface of the adrenals in 41 dogs multiple, small, nodular tumors of the fascicular cells of the cortex were found. In 39 the spleen was involved by 1 to 6 smooth, tense, round, deep purple tumors projecting above the surface and composed of densely packed masses of lymphocytes with large proliferating centers. Myelopoiesis was seen near these growths. Most of the spleens were affected by hemosiderosis. In 39 dogs the liver was the site of 3 to 20 small nodular tumors of liver cells. The prostates of 22 males were affected by benign enlargement. Situated in the testes were 17 benign growths including multiple interstitial cell tumors and adenomas of the tubules. In 11 dogs the thyroid was the seat of multiple tumors including cylindromatous, fetal-adenomatous, and massed cellular arrangements. Pancreatic adenomas of acinar origin were discovered in 5 dogs. In addition, 13 malignant neoplasms and a variety of benign tumors were observed. The high incidence of small, nodular tumors of the fascicular cells of the adrenal cortex; of the smooth, tense, round, deep purple tumors of lymphatic tissue in the spleen; of the small nodular tumors of liver cells; of benign hypertrophy of the prostate; of the interstitial cell tumors and tubular adenomas in the testes; and of the nodular tumors of varying architecture in the thyroid would indicate that these proliferations could be more properly designated nodular hyperplasias of these organs rather than true tumors. The growths of acinar origin in the pancreas might also be thought to belong among the nodular hyperplasias of parenchymatous viscera, particularly in the light of the statistics of Cohrs (3) and of Pallaske (14).

In the necrospy findings on 29 dogs examined by Cohrs (15 males, 13 females, and 1 of unmentioned sex, in 27 of which the ages ranged between 10 and 22 years), nodular hyperplasia of the adrenal cortex was present in 17; of the splenic follicles, in 15; of the liver cells, in 13; of the pancreas, in 13; and of the thyroid, in 5. Cohrs (3) called the nodules in the adrenal cortex "hypernephromas"; the tumors of the liver cells, "adenomas"; and the nodules in the thyroid, "strumas."

In his necropsy investigation of interstitial cell tumors (*"Zwischenzellentumoren"*) of the testis Pallaske studied 107 male dogs, of which 17 were 5 years of age or younger; 37, 6 to 9 years old; and 53, 10 to 20 years old. Of these 107 dogs, the 31 with so-called interstitial cell tumors of the testis were 6 to 20 years of age; 23 of the 31 were 10 to 20 years old. Seventy-one dogs, 6 to 20 years old, revealed nodular hyperplasia of one or more of 5 organs; namely, liver, adrenals, spleen, pancreas, and thyroid. Of these 71, 28 animals, 6 to 16 years old, also had so-called interstitial cell growths of the testes. From the evidence presented in these 4 papers the presence of one or more nodular "tumors" of the fascicular cells of the adrenal cortex, of the lymphatic tissue of the spleen, of the liver cells, of the acini of the pancreas, of the interstitial cells of the testes, and of the thyroid parenchyma may be suitably termed "nodular hyperplasias" rather than true neoplasms. Such cellular proliferations (including benign prostatic hypertrophy) found in dogs at routine necropsy should be accepted as internal evidences of senility of the animals just as surely as decay and loss of teeth, grayness of hair, and senile cataract are external evidences of canine old age. This is especially true if one recalls that such changes are preponderant in dogs 10 years of age or older, and rare in dogs under the age of 6 years.

A REVIEW OF THE LITERATURE

In the following summary from the literature of multiple tumors in dogs nodular hyperplasias as defined will be omitted when they have been found in the protocols of cases reviewed. This procedure has resulted in the rejection of some of the cases described by Cohrs as instances of multiple tumors.

Virchow (19) described a bitch with: (a) a large ossifying enchondroma of the breast with ossifying metastases of fibrous hyaline cartilage in the lungs, pleura, and mediastinum; and (b) a large, centrally cystic tumor of the omentum.

van Gieson (18) reported the case of a female adult dog afflicted by : (a) intracanalicular fibromas of the right penultimate and hindermost mammae, (b) a telangiectatic sarcoma of mesenteric lymph nodes, (c) 2 myomas of the stomach, (d) a fibroma of the uterus, and (e) a follicular cyst of the left ovary.

Petit (15) recorded the case of a female dog with: (a) mammary carcinoma with generalized metastases, (b) multiple fibromas of the vagina, and (c) ovarian cysts.

Case 4 of Ortschild (13) was an 8 year old Gordon setter bitch in which were found: (a) an adenocarcinomatous mixed tumor of the right hindermost mamma, and (b) a lipoma of the left pectoral region.

In a male St. Bernard dog 12 years old Cullen (4) discovered: (a) a recurrent squamous cell carcinoma of the skin of the back, and (b) an adenocarcinoma of the prostate.

Heney and Wooldridge (9) discussed the case of an aged female dachshund with: (a) a lipoma of the perineum, and (b) a fibroma of the right penultimate mamma.

Winokuroff (20) described a 22 year old dog afflicted by: (a) a spindle cell sarcoma of the retroperitoneal connective tissue in the neighborhood of the left inferior costal arch, and (b) primary multiple cystadenomas of the liver.

Stephan (17) reported the case of a 10 year old female terrier with: (a) a mammary carcinoma with pulmonary metastases, and (b) a cavernous hemangioma of the liver.

Wooldridge (21) recorded the case of a male fox terrier 14 years old with: (a) adenoma of the prostate, and (b) adenocarcinoma of the liver.

Bartlett (2) reviewed the subject of multiple primary malignant tumors and included 2 cases occurring in dogs. The first dog was an old male afflicted with: (a) an adenocarcinoma of the thyroid with metastases to the lungs, mediastinum, and pineal region; (b) bilateral primary adrenal mesotheliomas showing early malignancy; (c) bilateral congenital multicystic kidneys; and (d) a cavernous hemangioma of the liver. The second dog was an old female with: (a) an adenocarcinoma of the thyroid with metastases to the cervical lymph nodes and lungs, and (b) a mixed tumor (chondrosarco-endothelioma) of the right caudal mamma.

Ball (1) described a 9 year old male poodle with: (a) a metatypic glandular epithelioma of the pancreas; and (b) a large round cell sarcoma of the duodenum with metastases to the omental lymph nodes, lungs, spleen, and kidneys.

Mettam (12) reported the case of a male pointer 17 years old with: (a) an adenocarcinoma of the anal sebaceous glands with metastases in the right lung, and (b) a spindle cell sarcoma of the ileocecal mesentery.

In an old male setter Goodpasture (7) found: (a) chronic lymphatic leukemia with the primary focus in the 2,730 gm. spleen; secondary foci in the thymus, mediastinum, and pelvis; and metastases in the brain, heart, and testis; and (b) 2 large carcinomas of the lung.

Joest (11) reported the case of a female St. Bernard dog 12 years old with (a) a polymorphous chondroblastic sarcoma of the pubic bone wtih metastases in the lungs, and (b) multiple fibrosarcomas of the vagina.

Scheunert (16) recorded the case of a dog 11 years old with: (a) a primary spindle cell sarcoma of the subcutis without metastases, and (b) a primary spindle cell sarcoma of the omentum with metastases.

Holtmann (10) described an old female dog with: (a) a primary sarcoma of the liver with metastases, and (b) a primary carcinoma of the breast.

Cohrs (3) reported several cases of multiple primary tumors in dogs. Summaries of the acceptable cases are as follows:

Case 1.—Male dachshund, 14 years old; (a) cutaneous melanoma with transformation to spindle cell sarcoma adjacent to the left eye, (b) 4 hard cutaneous papillomas of the neck and trunk; (c) an ulcerated, cornified, squamous cell carcinoma of the lumbar skin; (d) 3 lumbar sebaceous gland adenomas, (e) 2 adenomas of the circumanal glands; and (f) a papillary adenoma (originating from bronchial glands) of the right lung.

Case 2.—Male poodle, 12 years old; (a) adenoma of the circumanal glands, (b) subcutaneous hemangioma of the left hind leg, and (c) ulcerated leiomyoma of the ileum.

Case 3.—Male pinscher, 20 years old; (a) multiple leiomyomas of the stomach, and (b) 3 cystadenomas of the testes.

Case 5.—Male mongrel dachshund, 15 years old; (a) adenocarcinoma of the circumanal glands with pulmonary metastases, (b) leiomyoma of the spleen, and (c) medullary carcinoma of the right testis.

Case 6.—Female Doberman, 16 years old; (a) teratoid mixed tumor of the left hindermost mamma with chondrosarcomatous metastases to the regional lymph nodes and lungs, (b) teratoid mixed tumor of the right hindermost mamma, and (c) fibroma of the spleen.

Case 7.—Female boxer, 11 years old; (a) 2 subcutaneous fibrolipomas, (b) 2 subcutaneous lipomas, (c) a subcutaneous fibroma, and (d) teratoid mixed tumors of both third mammae and of the right fifth mamma.

Case 8.—Female French bulldog, 13 years old; (a) medullary carcinoma of the right lobe of the liver with metastases to other liver lobes, the gall bladder, the portal lymph nodes, the diaphragm, and the lungs; (b) an epulis adjacent to the left upper canine tooth; (c) a leiomyoma of the stomach; and (d) 5 leiomyomas of the urinary bladder.

Case 9.—Female dachshund, 14 years old; (a) fibrosarcoma of the lower jaw, and (b) adenocarcinoma of the left hindermost mamma.

Case 11.—Female fox terrier, 14 years old; (a) teratoid mixed tumor of the right first mamma, and (b) carcinoma of the thyroid gland with pulmonary metastases.

Case 12.—Male pinscher, 14 years old; (a) multiple hard cutaneous papillomas, and (b) solid carcinoma of the liver with hepatic metastases.

Case 13.—Female sheep hound, 12 years old; (a) recurrent mixed cell sarcoma of the third left mamma, (b) teratoid mixed tumor of a right mamma, and (c) adenocarcinoma of the liver with pulmonary metastases.

Case 14.—Male pinscher, 5 years old; (a) periosteal mixed cell sarcoma of the left foreleg with pulmonary metastases of large round cell sarcoma, and (b) a medullary carcinoma of the left testis.

Case 17.—Male German sheep hound, 14 years old; (a) 2 leiomyomas of the stomach, and (b) a leiomyoma of the gall bladder.

Case 18.—Female pinscher, 15 years old; (a) sebaceous adenoma of the left hind leg, (b) 5 mammary adenomas, (c) an adenocarcinoma of the liver with metastases in the liver and portal lymph nodes.

Case 19.—Female fox terrier, 14 years old; (a) a sebaceous adenoma of the left hind leg, and (b) a papillary adenoma (originating from bronchial glands) of the left lung.

Case 21.—Male Rehpinscher, 16 years old; (a) leiomyoma of the gall bladder, (b) a medullary carcinoma in each testis, and (c) a medullary carcinoma of the thyroid gland.

Case 22.—Female pinscher, 11 years old; (a) subcutaneous hemangioma of the right thoracic wall, (b) a hard papilloma of the vulva, and (c) an adenocarcinoma of the right third mamma with metastases in the neighboring axillary lymph nodes.

Case 23.—Female fox terrier, 17 years old; (a) adenomas of the last left and the right third mammae; (b) a subcutaneous, large, round cell sarcoma of the right thoracic wall; (c) 5 subcutaneous lipomas of the trunk; (d) a polypoid fibroma of the internal os of the uterus.

Case 24.—Female mongrel Doberman pinscher, about 14 years old; (a) leiomyoma of the pylorus, and (b) 7 spindle cell sarcomas of the vagina.

Case 25.—Male fox terrier, 16 years old; (a) an adenoma of the circumanal glands, (b) a carcinoma of the liver with hepatic metastases, and (c) a lipoma of the spleen.

Case 26.—Female pug dog, 13 years old; (a) multiple pigmented cutaneous nevi, (b) subcutaneous lipoma of the right thoracic wall, (c) adenoma of the circumanal glands, (d) leiomyoma of the esophagus, and (e) a polypoid fibroma of the vagina.

Case 27.—Male hunting dog, 16 years old; (a) ulcerated, cornified, squamous cell carcinoma of the right cervical region; (b) adenocarcinoma of the left lung; and (c) squamous cell carcinoma of the urinary bladder.

Case 28.—Old dachshund of unmentioned sex; (a) ossifying, periosteal, round cell sarcoma of the left foreleg, and (b) carcinoma of the thyroid with metastases to the lungs.

Case 29.—Male fox terrier, 10 to 15 years old; (a) subcutaneous lipoma of the left thoracic wall, (b) a carcinoma simplex of the left testis, and (c) a papillary adenoma of the left lung.

Case 30 (surgical).—Female dog, 3 years old; (a) a subcutaneous lipoma in the neck, and (b) a fibroma of the vagina.

Case 31 (surgical).—Male spaniel, 9 years old; (a) squamous cell carcinoma of the foot, and (b) adenoma of the circumanal glands.

Feldman (6) described a male shepherd dog 13 years old with: (a) a squamous cell carcinoma of the gum over the left side of the mandible, with metastases to a left submaxillary lymph node; (b) multiple cavernous hemangiomas of the liver; (c) a leiomyosarcoma of the cecum; and (d) a papillary cystadenoma of the prostate.

In their investigation of the canine prostate in relation to normal and abnormal testicular changes, Zuckerman and McKeown (22) studied the prostates and testes of 243 dogs, among which was a fox terrier (Case 225) 6 years old that had: (a) an adenocarcinoma of the testis, and (b) a carcinoma of the prostate.

Curth and Slanetz (5) described acanthosis nigricans in a female, part collie, German shepherd dog, 14

years old, that also had: (a) a carcinoma of the liver, and (b) a subcutaneous lipoma.

TABLE I: BREEDS OF 37 DOGS

Pinscher (various types)	8
Fox terrier	7
Dachshund	5
Setter	2
St. Bernard	2
Poodle	2
Sheep hound	2
Shepherd	2
Pointer	1
Doberman	1
Boxer	1
Bulldog	1
Hunting dog	1
Spaniel	1
Terrier	1
TOTAL	37

TABLE II: TYPES OF NEOPLASMS

Carcinoma	36
Sarcoma	21
Adenoma	14
Leiomyoma	11
Fibroma	9
Lipoma	9
Hemangioma	5
Mixed tumor	4
Cyst	4
Papilloma	3
Cystadenoma	3
Mesothelioma	1
Epulis	1
Melanoma	1
Fibrolipoma	1
TOTAL	123

In an analysis of the 46 cases of multiple primary tumors in dogs found in the literature, the following statements may be made concerning age and sex. The ages were known in 36; 2 were under 6 years, 4 were 6 to 9 years, 29 were 10 to 20 years, and 1 was over 20 years old. Of the 43 animals in which sex was mentioned, 23 were females and 20 were males. In Table I are listed the breeds of the 37 dogs in which the breed was stated. The 123 tumors found in the 46 cases included 58 malignant and 65 benign neoplasms. In Table II are summarized the various types of tumors represented. In Table III the primary sites of these 123 neoplasms are tabulated. Table IV contains the various combinations of malignant and benign neoplasms encountered in the 46 cases.

TABLE III: PRIMARY SITES OF 123 NEOPLASMS

Primary site	Total tumors	Malignant tumors	Benign tumors
Breast	18	10	8
Subcutaneous tissue	14	2	12
Skin	12	5	7
Liver	12	8	4
Circumanal glands	7	2	5
Testes	6	5	1
Thyroid	5	5	0
Stomach	5	0	5
Vagina	5	2	3
Lungs	5	2	3
Prostate	4	2	2
Spleen	4	1	3
Bone	3	3	0
Buccal cavity	3	2	1
Omentum	2	1	1
Uterus	2	0	2
Ovaries	2	0	2
Small intestine	2	1	1
Urinary bladder	2	1	1
Gall bladder	2	0	2
Adrenals	1	1	0
Kidneys	1	0	1
Pancreas	1	1	0
Large intestine	1	1	0
Mesentery	1	1	0
Esophagus	1	0	1
Lymph nodes	1	1	0
Retroperitoneum	1	1	0
TOTAL	123	58	65

TABLE IV: COMBINATIONS OF MALIGNANT AND BENIGN NEOPLASMS

1 malignant, 1 benign	11
1 " , 2 "	7
1 " , 3 "	2
1 " , 4 "	1
2 "	11
2 " , 1 benign	3
2 " , 2 "	2
2 " , 4 "	1
3 "	1
2 benign	5
4 "	1
5 "	1
Total	46

SUMMARY

The cases of multiple canine tumors recorded in the literature have been surveyed in order to determine the age, sex, and breed of dogs afflicted and the types and sites of the neoplasms encountered. The various combinations of malignant and benign neoplasms encountered in the 46 cases have been tabulated also.

REFERENCES

1. BALL, V. Pancreatic Epithelial and Intestinal Conjunctive Cancer in a Dog. Am. Vet. Rev., **46**:499-450, 1914-15. Abst. from Journ. de Zootchn.
2. BARTLETT, F. K. Multiple Primary Malignant Tumors—with a Report of Two Cases in Dogs. Arch. Int. Med., **13**:624-639. 1914.
3. COHRS, P. Über primäre Multiplizität von Geschwülsten bei Haustieren. Zeitschr. f. Krebsforsch., **24**:156-221. 1926.
4. CULLEN, E. K. A Case of Multiple Tumors in a Dog. Bull. Johns Hopkins Hosp., **18**:101-102. 1907.
5. CURTH, HELEN O., and SLANETZ, C. A. Acanthosis Nigricans and Cancer of the Liver in a Dog. Am. J. Cancer, **37**:216-223. 1939.
6. FELDMAN, W. H. Multiple Primary Neoplasms in Lower Animals. Am. J. Path., **4**:497-506. 1928.
7. GOODPASTURE, E. W. An Anatomical Study of Senescence in Dogs with Especial Reference to Relation of Cellular Changes of Age to Tumors. J. M. Research, **38**:127-190. 1918.
8. GOODPASTURE, E. W., and WISLOCKI, G. B. Old Age in Relation to Cell-Overgrowth and Cancer. J. M. Research, **33**:455-474. 1916.
9. HENEY, F. A., and WOOLDRIDGE, G. H. Lipoma of the Perinaeum and Fibroma of the Mammary Gland of a Bitch. Veterinary Journal, **63**:465-466. 1907.

10. Holtmann, P. Beiträge zu den malignen Tumoren bei Hunden. Inaug.-Diss. Hannover 1922 (Auszug). Quoted by Cohrs.
11. Joest, E. Über ein metastasenbildendes chondroblastisches Sarkom des Schambeins beim Hunde. Zugleich ein Beitrag zum Auftreten zweier selbständiger Sarkomformen bei ein und demselben Individuum. Ber. Tierärtzl. Hochsch., Dresden. S. 119. 1918. Quoted by Cohrs.
12. Mettam, A. E. Two Different Malignant Growths in the Same Subject—A Dog. J. Comp. Path. and Therap., **28**: 49-51. 1915.
13. Ortschild, J. F. A Report of Eight Cases of Canine Neoplasm. Bull. Johns Hopkins Hosp., **16**:186-196. 1905.
14. Pallaske, G. Beitrag zur Frage der "Zwischenzellentumoren" bei Tieren. Virchow's Arch. f. path. Anat., **281**:856-870. 1931.
15. Petit, G. Fibromes multiples du vagin avec métrite chronique et kystes de l'ovaire chez une chienne. Bull. de la soc. centr. de méd. vét., **56**:237. 1902. Quoted by Cohrs.
16. Scheunert, M. Beitrag zur Kenntnis der Sarkomatose beim Hunde. Inaug.-Diss. Leipzig 1920. Quoted by Cohrs.
17. Stephan, L. Die Tumoren in der Leber des Hundes. Inaug.-Diss. Giessen 1909. Quoted by Cohrs.
18. van Gieson, I. J. Multiple Tumors in a Bitch. Proc. New York Path. Soc., **6**:80-81. 1887.
19. Virchow, R. Pathologie des Tumeurs. Paris, Germer-Bailliere, 1867-1876, vol. 1, p. 254. Quoted by Sutton, J. B. Tumours in Animals. J. Anat. and Physiol., **19**: 415-475. 1884-85.
20. Winokuroff, E. Einige seltenere Geschwülste bei Tieren. Inaug.-Diss. Bonn 1908. Quoted by Cohrs.
21. Wooldridge, G. H. Adenoma of the Prostate and Adenocarcinoma of the Liver in a Dog. Am. Vet. Rev., **42**: 223. 1912-13. Abst. from Journ. of Comp. Path. and Therap.
22. Zuckerman, S., and McKeown, T. The Canine Prostate in Relation to Normal and Abnormal Testicular Changes. J. Path. & Bact., **46**:7-19. 1938.

Antifibromatogenic Effects Produced by the Intermittent Action of Progesterone*†

Rigoberto Iglesias, M.D., Alexander Lipschütz, M.D., and Guillermo Nieto, M.D.**

(From the Department of Experimental Medicine, National Health Service of the Republic of Chile, Santiago, Chile)

(Received for publication February 24, 1944)

Abdominal fibroids and other toxic reactions induced by estrogens can be prevented by the simultaneous administration of progesterone; production of progesterone or other antifibromatogenic steroids might then tentatively be interpreted as a means of a bodily autodefense (1). But secretion of progesterone in the ovary is an intermittent phenomenon; so the question arises whether abdominal fibroids due to a prolonged administration of fibromatogenic quantities of estrogens can be prevented also by an *intermittent* action of progesterone as was the case with the *continuous* one in our former experiments.

Tablets of estradiol dipropionate were implanted subcutaneously into castrated female guinea pigs; tablets of progesterone also were implanted into these animals 10 days later but withdrawn after another 10 days, to be afterwards implanted again, and so forth (see Fig. 1). The progesterone tablets were prepared from a mixture containing 40 per cent of progesterone and 60 per cent of cholesterol; they weighed 63 to 128 mgm. They were 5 mm. in diameter and several mm. in thickness; repeated withdrawal without breakage was facilitated by their volume, form, and consistency.

The fibromatogenic effect was classified according to the rules explained in a former paper (2), and was compared with that obtained in a group of animals in which tablets of estradiol dipropionate alone were implanted.

As may be seen from Table I all animals with estradiol dipropionate alone, acting for 103 to 108 days or less, had abdominal fibroids with an average fibrous tumorous effect (F.T.E.) of 7.2. There were large uterine, apical, mesenteric, and splenic tumors. Seven out of 12 animals surpassed the average F.T.E. On the contrary, there was in the estradiol-progesterone group (50 days of simultaneous action of estradiol and progesterone, and 53 to 56 days of estradiol alone) not a single animal that would have reached the average of the estradiol group. The number of animals with tumors of classes 2 and 3 (about 3 to 6 mm. in diameter or more) dropped greatly. The average F.T.E. in the estradiol-progesterone group was only 2.5. The significant difference between the two groups was more than 5.

The preventive effect of the discontinuous action of progesterone became manifest also with respect to the uterus. As seen from the table the uterine weight in the estradiol-progesterone group was considerably smaller than in the estradiol group.

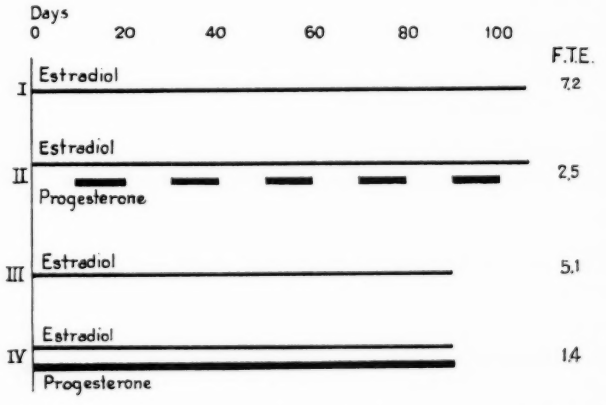


FIG. 1.—Comparative results with discontinuous (I-II) and continuous (III-IV) action of progesterone against estradiol. In the continuous progesterone group quantities of the steroid were absorbed that were near the antifibromatogenic threshold; in the discontinuous group quantities several times greater were used. Prevention of abdominal fibroids was effective in both cases, but was less complete with the discontinuous action. This might have been partly due to the greater duration of the experiments in the discontinuous group.

F.T.E.—average fibrous tumoral effect of the respective group.

The results above leave no doubt that abdominal fibroids induced by estrogen can be prevented by the intermittent action of progesterone, as by its continuous action, but the prevention was less complete. For the sake of comparison we may refer to a group of 14 animals that were under the simultaneous and continuous action of estradiol and small quantities of progesterone for 90 days (3; see Table II, Group Ib;

* This investigation has been aided by grants from The Jane Coffin Childs Memorial Fund for Medical Research and The Rockefeller Foundation.

† Thanks are due to Dr. Karl Miescher, in Basel, and Dr. E. Oppenheimer, of Ciba Pharmaceutical Products, in Summit, N. J., for estrogens and progesterone.

** Working in this Department for his M. D. thesis. Presented in the Faculty of Medicine of Bogotá (Colombia).

also Fig. 1 of the present paper). The quantities of progesterone absorbed daily during the discontinuous progesterone periods (50 days) were 2 to 3 times greater than the quantities absorbed daily in the continuous progesterone group during 90 days in the taneously implanted tablets of estradiol can be prevented by progesterone, even when the antifibromatogenic steroid is allowed to act only intermittently.

These findings support the concept that the rhythmic secretion of progesterone in the ovary is a means of

TABLE I

Upper part: Castrated female guinea pigs under continuous action of estradiol dipropionate during 103 to 108 days. Lower part: Discontinuous interaction of progesterone—10 days free of progesterone, and 10 days with progesterone, etc.

No.	Estradiol absorbed daily * during 103 to 108 days, μgm.	Progesterone absorbed daily during 50 days, μgm.	Weight of uterus, gm.	Fibrous tumorous effect (F.T.E.), Total units †	Class of tumors ‡			
					Uterine	Apical	Dig. tract and abd. wall	Spleen
14	31	0	7.0	**2.5**	0.5pm.	1	0.5f.	0.5s.
8N	42	0	3.0	**2.5**	0	2	0.5u.bl.	0
2N	38 §	0	4.0	**5.0**	0	3	0	2
13	40	0	4.8	**6.5**	0.5pm.	3	3ms.	0
18	36	0	4.0	**6.5**	0	3	3ms.	0.5fs.
42N	27 ‖	0	5.6	**8.0**	2pm.	2	2ms.	2
10N	—	0	12.0	**8.0**	2ss., pm.	1	3ms., d.	2
17	39	0	4.3	**8.5**	2ss., pm.	3	3ms.	0.5f.
11	34	0	—	**9.0**	2pm.	3	3ms., pr.	1
12	42 *	0	8.2	**9.5**	3pm.	3	3ms.	0.5s.
15	41	0	—	**9.5**	3pm., ss.	3	3ms., d.	0.5s.
16	34	0	—	**11.0**	3pm.	3	3ms., d.	2
8	46	29	4.9	**1.0**	0	0	0.5f.	0.5f.
2	33	25	2.5	**1.0**	0	0	0.5f.	0.5fs.
10	40	56	2.5	**1.5**	0	0	0.5f.	1
11N	51	—	4.7	**1.5**	1ss.	0	0	0.5s.
5	39	46	2.5	**2.0**	0.5ss.	0	0.5fs.	0.5f.
4	40	32	2.2	**2.5**	0	2	0.5f.	0
3	40	39	4.1	**2.5**	0	0.5f.	1fs.	1fs.
9	42	29	3.7	**3.0**	0	2	0.5fs.	0.5f.
1	44	25	4.2	**3.0**	0	2	0.5f.	0.5fs.
6	48	36	5.5	**4.5**	1ss.	2	1s.	0.5s.
7	56	67	3.5	**5.0**	2pm.	0.5fs.	2d.	0.5fs.

* Free estradiol; the weight of estradiol dipropionate divided by 1.41.

† The F.T.E. is the sum of the figures of the following four columns.

‡ For the system of classification see (2).

§ 74 days only ‖ 90 days only.

Abbreviations: f = fibrous strands; s = accumulation of small tumorous nodules ("tumorous seed"); fs. = fibrous strands and "seed" together present; pm. = parametric tumors; ss. = subserous tumors of the uterus; ms. = mesenteric tumors; pr. = tumors of the peritoneum; d. = tumors of the diaphragm; u.bl. = tumor of the urinary bladder.

earlier experiments of Lipschütz and others. This gives evidence that the less complete prevention in the discontinuous group was not due to unfavorable quantitative conditions. It might have been due partly to the greater duration of the experiments in the discontinuous progesterone group. But one may also tentatively suggest that the antifibromatogenic effect depends also upon the time of the progesterone action in relation to the time of the fibromatogenic action of the estrogen.

SUMMARY

Uterine and other abdominal fibroids induced in the female guinea pig by the prolonged action of subcutaneously implanted tablets of estradiol can be prevented by progesterone, even when the antifibromatogenic steroid is allowed to act only intermittently.

These findings support the concept that the rhythmic secretion of progesterone in the ovary is a means of bodily autodefense against the toxic and tumor-producing actions of estrogens.

REFERENCES

1. LIPSCHÜTZ, A. Endocrine Aspects of Antitumoral Autodefense. Rev. canad. de Biol., **2**:92-104. 1943.
2. LIPSCHÜTZ, A., BELLOLIO, P., CHAUME, J., and VARGAS, L., JR. Comparative Conjunctive Tumorigenic Action of Three Different Esters of Estradiol. Proc. Soc. Exper. Biol. & Med., **46**:164-167. 1941.
3. LIPSCHÜTZ, A., BRUZZONE, S., and FUENZALIDA, F. Comparative Antifibromatogenic Activity of Progesterone and Related Artificial Steroids. Cancer Research, **4**:179-185. 1944.

Inactivation of Antifibromatogenic Substances (Progesterone and Desoxycorticosterone Acetate) in the Liver

Christiane Dosne, Ph.D.*†

(From the Department of Experimental Medicine, National Health Service of the Republic of Chile, Santiago, Chile)

(Received for publication March 15, 1944)

INTRODUCTION

It is a well-known fact that estrogens (the fibromatogenic substances) are destroyed in the liver, while it is still debatable whether such hormones as progesterone and desoxycorticosterone (the antifibromatogenic substances) have a similar fate when they reach this organ. Fels and Mónaco (6) found that progesterone, whether injected under the skin, intraperitoneally, or directly into the liver, the spleen, or the uterus, gave the same progestational threshold in the rabbit. These authors concluded that, unlike the estrogens, progesterone is not inactivated by the liver. Eversole and Gaunt (5) found no significant difference in growth and survival between adrenalectomized rats with desoxycorticosterone acetate (DCA) pellets implanted into the spleen, and their various controls with pellets implanted under the skin, in the kidney, or intraperitoneally. Mark (14) obtained the same decrease of sodium chloride intake in adrenalectomized rats with DCA pellets placed under the skin or in the spleen. Accordingly these authors concluded that there is no evidence that DCA is inactivated by the liver or spleen. Burrill and Greene (3), on the other hand, found in adrenalectomized rats with subcutaneously and intramesenterically implanted pellets that the liver can inactivate DCA but that this ability is quantitatively limited, since with greater quantities of the hormone, absorbed from larger intramesenterically implanted pellets, survival and growth of adrenalectomized rats was observed.

Progesterone and desoxycorticosterone acetate administered subcutaneously are two of the main antifibromatogenic substances (9, 12, 13). Thus it was thought that placing tablets of these substances in the spleen, that is, draining them directly into the portal circulation, should help to solve the problem under discussion. Bruzzone and Cuevas (2) obtained complete antifibromatogenic action with large tablets of progesterone placed in the liver. However, in view of a possible quantitative limitation in liver inactivation similar to that observed for the fibromatogenic action of estrogens in this laboratory (1, 4), it seemed profitable to perform the following experiments, using various quantities of progesterone and of DCA, administered intrasplenically.

EXPERIMENTS

The method employed for obtaining a fibromatogenic reaction was the same as has been reported before (8). An α-estradiol tablet (the fibromatogenic substance) was placed under the skin of castrated female guinea pigs of approximately 300 gm. body weight (11). In these same animals a tablet of progesterone or of DCA (the antifibromatogenic substances) was inserted well into the spleen (Groups III and IV in Table I). Animals to be used as controls were treated exclusively with the fibromatogenic substance (Group I), while others received both the fibromatogenic and antifibromatogenic substances, both, however, implanted subcutaneously (Groups II and IV).

In an effort to obtain a wide range of absorption all sizes of tablets were used, while some of the tablets were made up of 60 per cent cholesterol and 40 per cent of the desired substance, as it has been proved that the absorption is not selective (7, 9). The experiments lasted 90 to 96 days at the end of which time the animals were autopsied. The amount of hormone absorbed was calculated from the loss of weight of the tablet cleaned of its fibrous capsule, washed, and dried in a vacuum. The results are summarized in Table I.

The fibrous abdominal reaction was classified according to the rules explained in former papers (9, 10): the total, or fibrous tumorous, effect (F.T.E.), averaging somewhat more than 5 in the control group (I) and decreasing proportionally with the antifibromatogenic action; the number of animals within each group reaching the control average F.T.E. of 5; finally,

* Under a grant in aid given by the Rockefeller Foundation to the author during her stay in Chile.

† This work has been aided by The Jane Coffin Childs Memorial Fund for Medical Research and the Rockefeller Foundation. Grants administered by Professor A. Lipschütz.

TABLE I: COMPARATIVE ANTIFIBROMATOGENIC EFFECT OBTAINED WITH SUBCUTANEOUS OR INTRASPLENIC IMPLANTATION OF PROGESTERONE AND DESOXYCORTICOSTERONE ACETATE TABLETS IN 71 CASTRATED FEMALE GUINEA PIGS SIMULTANEOUSLY RECEIVING SUBCUTANEOUS IMPLANTATION OF TABLETS OF α-ESTRADIOL

Group No.	Estradiol absorbed daily, μgm.	Antifibromatogen administered	Mode of administration of the antifibromatogen	Absorption of the antifibromatogen daily, μgm.	Fibrous tumorous effect (F.T.E.), average	Number of animals		Average of tumorous marks, classes 2 and 3, per animal
						Total	Reaching F.T.E.=5	
I	6-21	0	0	0	5.2	10	5	1.5
IIa	17-33	Progesterone	Subcutaneously	12-30	**1.1**	6	0	0
IIb	13-31	"	"	59-164	1.4	4	0	0
IIIa	25-97	"	Intrasplenically	16-29	**4.0**	8	2	1.1
IIIb	18-39	"	"	33-57	2.8	9	1	0.7
IIIc	24-78	"	"	85-148	2.7	9	1	0.2
IVa	19-134	Desoxycorticosterone	Subcutaneously	9-36	5.0	4	2	1.7
IVb	23-126	acetate	"	40-153	**1.1**	6	0	0
Va	13-92	"	Intrasplenically	9-36	4.6	16	7	1.3
Vb	17-72	"	"	40-131	**4.3**	9	3	1.2

the incidence of individual tumorous values of classes 2 and 3 per animal (tumors of about 3 mm. in diameter or more).

It can be seen, as has been repeatedly observed (9), that in the present work the antifibromatogenic threshold, or the amount necessary to prevent abdominal fibroids, was again approximately 12 to 30 μgm. daily for progesterone (Group IIa). The threshold dose was more than 40 μgm. a day for DCA (Group IVb): there was no antifibromatogenic action with less than 40 μgm. of DCA daily (Group IVa).

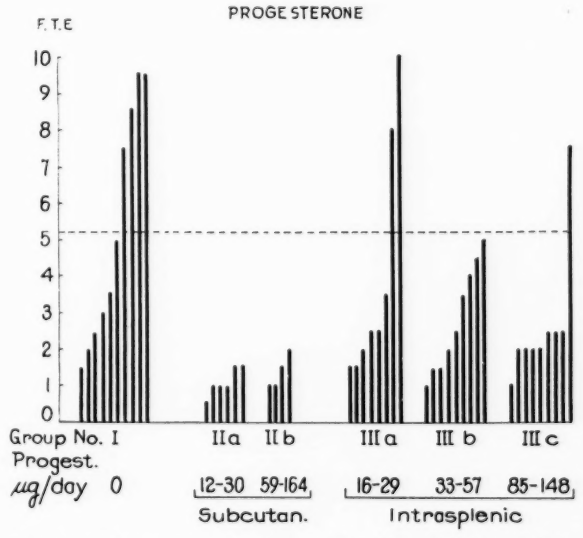


FIG. 1.—Fibromatogenic effect with α-estradiol alone (Group I), with α-estradiol and subcutaneously implanted progesterone (Groups IIa and IIb), and with α-estradiol and varying doses of intrasplenically implanted progesterone (Groups IIIa, IIIb and IIIc). Each column corresponds to an individual experiment. The dotted line indicates the average F.T.E. of the control animals (Group I) receiving only α-estradiol.

Progesterone, administered intrasplenically in doses of 16 to 29 μgm. per day, was not able to prevent the appearance of abdominal fibroids (Group IIIa), as the F.T.E. observed was almost as high as in the control group without progesterone. With larger doses some antifibromatogenic action was obtained, as judged by the lower F.T.E. (Groups IIIb and IIIc); but even with these greater quantities of progesterone the antifibromatogenic action was never as distinct as with subcutaneous administration (Group IIb). These differences between the various progesterone groups can be clearly appreciated in Fig. 1, where individual results (F.T.E.) are graphically represented.

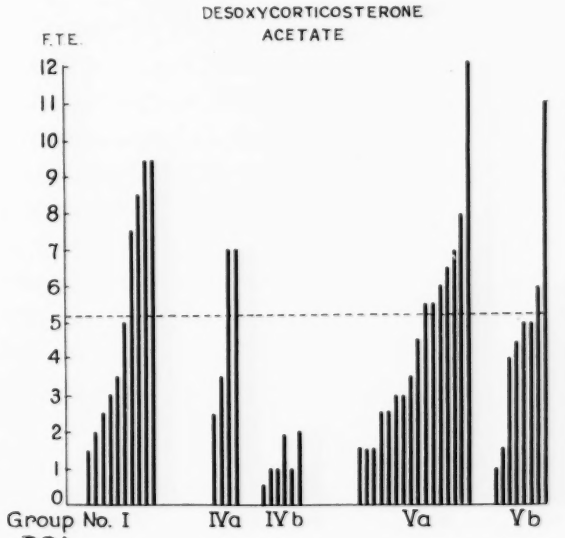


FIG. 2.—Fibromatogenic effect with α-estradiol alone (Group I), with α-estradiol and varying doses of subcutaneously implanted DCA (Groups IVa and IVb), and with α-estradiol and varying doses of intrasplenically implanted DCA (Groups Va and Vb).

DCA, administered intrasplenically in small as well as in comparatively large doses, did not prevent the appearance of abdominal fibroids (Group V). It can be seen that animals with intrasplenically implanted tablets of DCA, with an absorption of large doses (Group Vb), behaved similarly to animals with an absorption of doses not reaching the antifibromatogenic threshold (Groups IVa and Va). The individual F.T.E. figures are given in Fig. 2.

Thus we can deduce from these observations that progesterone as well as desoxycorticosterone acetate passing directly through the liver are inactivated, as judged by the decreased antifibromatogenic effect in those groups in which these substances were absorbed from intrasplenically implanted tablets (Groups IIIa and Vb with a F.T.E. similar to animals with estradiol only). But at the same time our observations show that above a certain dose a portion of the hormone escapes inactivation, as judged by the lowering of the F.T.E. in Groups IIIb and IIIc, compared with Groups I and IIIa.

SUMMARY

The liver is capable of inactivating antifibromatogenic substances such as progesterone and desoxycorticosterone acetate, but this ability is a quantitatively limited one.

I am greatly indebted to Professor A. Lipschütz, under whose supervision this work was performed, and to Dr. R. Iglesias for advice during necropsies.

REFERENCES

1. Acuña, L. Injerto intraesplénico comparativo del estradiol y de sus ésteres. Tesis Universidad de Chile, 1942 (Public. Med. Exp., No. 12).
2. Bruzzone, S., and Cuevas, F. Unpublished experiments.
3. Burrill, M. W., and Greene, R. R. Capacity of Rat's Liver to Inactivate Desoxycorticosterone Acetate. Endocrinology, **30**:142-145. 1942.
4. Carrasco, R. Influencias hepáticas en la tumorigenesis inducida por estrógenos. Tesis Universidad de Chile, 1943 (Public. Med. Exp., No. 18).
5. Eversole, W. J., and Gaunt, R. Desoxycorticosterone: A, its Loss in the Digestive Tract, B, Effect on Water Intoxication. Am. J. Physiol., **133**:271. 1941.
6. Fels, E., and Mónaco, H. La acción de la progesterona administrada por distintas vías. Rev. Soc. argent. de biol., **17**:385-388. 1941.
7. Fuenzalida, F. Sobre la absorción de mezclas de esteroides activos y colesterol. Rev. Med. y Alim. (Chile). In press.
8. Iglesias, R. Tumores experimentales uterinos y extragenitales provocados por el benzoato de estradiol. Tesis Universidad de Chile, 1938 (Public. Med. Exp. No. 1).
9. Lipschütz, A., Bruzzone, S., and Fuenzalida, F. Comparative Antifibromatogenic Activity of Progesterone and Related Artificial Steroids. Cancer Research, **4**:179-185. 1944.
10. Lipschütz, A., and Maas, M. Progesterone Treatment of Uterine and Other Abdominal Fibroids Induced in the Guinea Pig by Alpha-Estradiol. Cancer Research, **4**: 18-23. 1944.
11. Lipschütz, A., and Vargas, L., Jr. Experimental Tumorigenesis with Subcutaneous Tablets of Œstradiol. Lancet, **1**:1313-1318. 1939.
12. Lipschütz, A., Vargas, L., Jr., and Nuñez, C. Comparative Antitumoral Action of Desoxycorticosterone Acetate and Testosterone Propionate. Proc. Soc. Exper. Biol. & Med., **48**:271-274. 1941.
13. Lipschütz, A., Vera, O., and González, S. The Relation of the Antifibromatogenic Activity of Certain Steroids to Their Molecular Structure and to Various Actions of These Hormones. Cancer Research, **2**:204-209. 1942.
14. Mark, J. Inactivation of Desoxycorticosterone Acetate. Endocrinology, **31**:582-585. 1942.

An Experimental Study of the Lateral Spread of Epidermoid (Squamous Cell) Carcinoma in Man, and the Reaction of Such a Lesion to the Wound-Healing Stimulus*

Alexander Brunschwig, M.D., and Thomas F. Thornton, Jr., M.D.

(From the Department of Surgery, The University of Chicago, Chicago 37, Illinois)

(Received for publication March 27, 1944)

Previous experimental studies (1) in mice and histologic studies of specimens from human patients were interpreted to suggest that squamous cell carcinoma arises by malignant transformation of a segment of squamous epithelium and not as a result of such changes occurring in one cell or a small nidus of cells. Furthermore, the experimental evidence recorded was interpreted by the authors to indicate that while the principal factor in the increase in size of such lesions was the increased number of cells resulting from continuous mitotic activity another factor, namely, progressive malignant change in the normal epithelium immediately apposed to the malignant epithelium, also contributed to lateral spread of the process. This hypothesis is not a new one (Ribbert, Adami) and admittedly has few adherents today. Still another observation in the study just referred to, which was made on mice bearing methylcholanthrene squamous cell cutaneous carcinomas, was that when the carcinoma was bisected, one-half of it removed, and normal skin approximated to the cut edge of the growth by suture with subsequent spontaneous separation of the wound to produce a chronic ulcer, healing of the latter was attempted by epithelial growth from the side of the normal epithelium and not from the margin composed of malignant cells. However, the neoplasm did increase in size in the direction *away* from the cut edge; that is, lateral spread went on over the continuously intact margins of the growth.

A patient presenting a squamous cell carcinoma of the dorsum of the left hand was recently observed, in whom an experiment similar to the last one mentioned above in mice was carried out. A summary of the history is as follows:

J.J.M. (67485) 62 years of age in 1932. Admitted September 9, 1932, with an ulcerating lingual carcinoma (biopsy) involving the right anterior margin, and rather extensive leukoplakia of the tongue. The Wassermann reaction was ++++. On December 2, 1932, the right anterior half of the tongue was resected. On March 6, 1933, a small ulceration in the wound was resected and found to be carcinoma. On April 19, 1937 a more radical partial glossectomy was performed for the third recurrence. He was seen at intervals until 1940, no recurrences being observed.

He was readmitted November 20, 1943, now 73 years of age, presenting an oval, fungating, and ulcerating lesion on the dorsum of the left hand, that had been present for a year and had been treated elsewhere by x-ray on several occasions. There was no evidence of recurrent carcinoma in the tongue or of lymph node metastases in the neck. Biopsy of the lesion on the hand revealed squamous cell carcinoma, and at the junction of the malignant and normal epithelium at the edge of the lesion a typical "zone of continuity" was present, *i.e.*, a narrow zone in which malignant and nonmalignant cells could not be morphologically differentiated.

The Wassermann reaction was still ++++ in spite of considerable antiluetic therapy. His mentality was impaired and he had been living a parasitical existence for several years, depending entirely upon a daughter who herself was now incapacitated because of multiple sclerosis.

On November 22, 1943, the lesion was bisected under local anesthesia and the usual aseptic surgical precautions, and one-half was removed together with a semicircular patch of skin. A fresh wound was thus created, one margin of which was composed of malignant epithelium (Figs. 1 to 4). Vaseline dressings were applied and the progress of the area was noted until January 31, 1944, when the whole area was excised. No metastases were palpable in epitrochlear or axillary nodes. Later, when the area of excision had partially healed, and required only a small dressing, the patient was discharged to an institution.

* This investigation was facilitated by the O. C. Miller Fund for Cancer Research of the University of Chicago.

FIG. 1.—Dorsum of left hand of J.J.M., day of operation. C, bisected squamous cell carcinoma; U, surgical ulcer; T, partially exposed extensor tendon; this is stationary marker to gauge closure of ulcer margins from normal skin edges.

FIG. 2.—Eighteen days after operation. Little change in size of carcinoma. The ulcer has become smaller because of healing from margin composed of nonneoplastic tissue. No tendency of malignant epithelium to cover ulcer.

FIG. 3.—Thirty-five days after operation. Further healing of ulcer from normal skin margins. Increase in size of carcinoma away from ulcer. No tendency of malignant neoplasm to extend over ulcer.

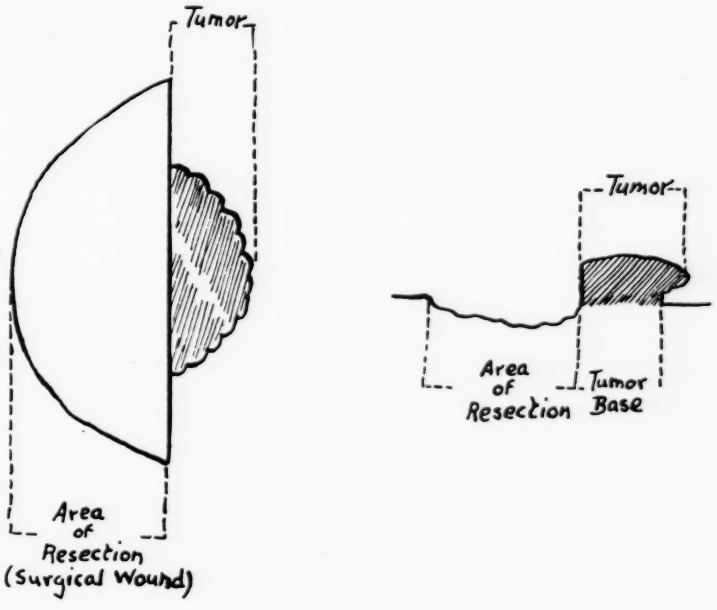
FIG. 4.—Fifty-three days after operation. The neoplasm has extended appreciably away from ulcer. The latter is almost healed by growth from nonneoplastic margins. No evidence of proliferation of malignant epithelium over ulcer; indeed, center of segment of tumor, N, bordering ulcer margin has become necrotic.

During the period of observation measurements of the flat, ulcerated area and of the neoplasm were made at intervals (Table I). After the 18th day the lesion exhibited fungations that overhung the actual base of its nontraumatized margins. Thus it appeared larger when the extent of these fungations was included in the measurements. In the Table the dimensions of the base and dimensions including the fungations are recorded separately.

decreased in size, healing taking place from the margins composed of nonneoplastic epithelium. A partially exposed extensor tendon (T in Figs. 1 to 4) served as a stationary marker in the subcutaneous tissues and study of the four accompanying illustrations shows the "healing in" of the ulcer from the normal skin margins.

Microscopic examination revealed typical squamous cell carcinoma. At the intact margins (Fig. 5) there

TABLE I AND DIAGRAM: DIAGRAMMATIC REPRESENTATION OF BISECTED SQUAMOUS CELL CARCINOMA ON DORSUM OF HAND OF PATIENT J.J.M. AND MEASUREMENTS OF TRAUMATIC ULCER AND ADJACENT NEOPLASM MADE AT INTERVALS FOLLOWING OPERATION



Date	Area of resection, ulcer		Area of tumor (base)		Area of tumor (including overhanging fungations)	
	Length	Breadth	Length	Breadth	Length	Breadth
Nov. 22-43	58	21 mm.	35	18 mm.		
Dec. 1-43 (8th day)	58	22 "	38	19 "		
" 10-43 (18th day)	48	23 "	41	18 "	41	28 mm.
" 22-43 (30th day)	30	18 "	40	20 "	40	28 "
" 30-43 (38th day)	26	15 "	40	24 "	40	28 "
Jan. 9-43 (48th day)	20	10 "	40	33 "	40	35 "
" 30-44 (69th day)	19	9 "	43	33 "	43	35 "

From the tabulated data it is seen that little change occurred in the size of the tumor and of the surgical ulcer during the 8 days following operation. By the 18th day increase in length of the lesion was noted and fungations had developed. Progressive increase in size then occurred. However, the spread was at the margins *not* traumatized by the operation. There was no evidence of even attempted spread of the malignant epithelium over the ulcer. Indeed, that portion of the tumor bordering the ulcer eventually became necrotic. From the eighth day onward the ulcer progressively

was the typical zone of continuity between malignant and normal epithelium. Sections through the tumor bordering the ulcer revealed downgrowth of columns of cells into the subcutaneous tissues and no apparent evidence of the mechanism that prevented these cells from growing over the chronic ulcerated area. Indeed, the histologic appearance was similar to that of the ulcerating border seen in routine biopsy sections of squamous epithelial lesions. The border of advancing normal epithelium was typical for the margins of a nonspecific ulcer.

DISCUSSION

The course of events in the human patient thus paralleled closely the observations previously reported in similar experiments with cutaneous squamous cell carcinomas produced by methylcholanthrene in mice; that is, the wound-healing stimulus obtained by the surgical excision of tissue did not stimulate locally the growth of the neoplasm. Similar conclusions were reached in studies with methylcholanthrene sarcomas in rats (2). Indeed, the malignant squamous epithelium appeared strikingly indolent toward such a stimulus, and seemed to have lost the property of purposeful proliferation to cover denuded areas. On the other hand, growth was progressive in areas that were undisturbed and the histologic findings here, as emphasized in a previous report, were consistent with the hypothesis (not, however, widely accepted) of progressive cancerization of normal cells as a factor in the lateral spread of squamous cell carcinomas. The intactness of the original junctional zone between malignant and nonmalignant squamous epithelium appeared to be a factor contributing to the lateral spread of this type of malignant process.

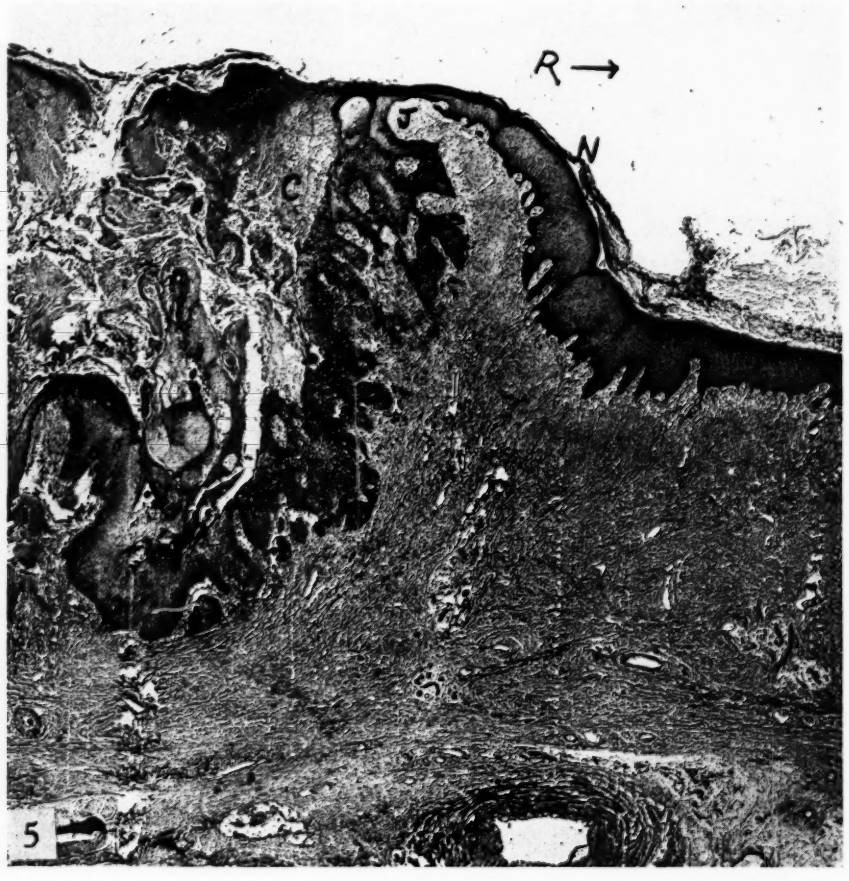


FIG. 5.—Section through intact advancing border taken at point S, Fig. 4, of lesion showing C, malignant epithelium; N, normal epithelium; J, junctional zone of continuity between normal and malignant epithelium. This junctional point has gradually progressed in direction R away from the wound, presumably as a result of progressive cancerization of normal epithelium immediately adjacent to malignant epithelium. Inasmuch as the carcinoma increased 15 mm. in width (see Table I) during the period of observation it appears that the junctional zone, J, shifted this distance during this period. Mag. × 15.

SUMMARY

Bisection of a squamous cell carcinoma in a human patient, with the production of a cutaneous defect adjacent to the portion of the tumor remaining *in situ* revealed: (a) lack of stimulation of the carcinoma along the incised margin; (b) healing of the cutaneous defect from the normal skin borders, with no evidence of retention of purposeful (healing) proliferation in the malignant epithelium; (c) continuous lateral spread of the carcinoma at the margins of the growth that were undisturbed by operative trauma.

REFERENCES

1. BRUNSCHWIG, A., and TSCHETTER, D. The Mode of Inception and Lateral Spread of Certain Squamous Cell Carcinomas. A Histopathologic and Experimental Study. Surg., Gynec. & Obst., **67**:715-721. 1938.
2. JULIAN, O. C., and BRUNSCHWIG, A. Reaction of Experimental Sarcomas to the Wound-Healing Stimulus. Surgery, **7**:32-36. 1940.

Abstracts

Experimental Research, Animal Tumors

Concentration of the Carcinogenic Material in a Venezuelan Spindle Oil by Simultaneous Molecular Distillation and Chromatographic Adsorption. Twort, J. M., and Lyth, R. [Christie Hosp., and Univ. of Manchester, England] *J. Hyg.*, **43**:248-251. 1944.

A fraction was obtained about 10 times as potent as the original oil.—I. H.

Factors Affecting Carcinogenesis. II. Incorporation of 3,4-Benzpyrene in Media Containing Purified Lecithin or Cephalin. Weil-Malherbe, H., and Dickens, F. [Royal Victoria Infirmary, Newcastle upon Tyne, England] *Cancer Research*, **4**:425-429. 1944.

Four groups of mice were given subcutaneous implantations or injections of a single dose of 0.3 mgm. of 3,4-benzpyrene dissolved in (a) purified lecithin; (b) purified cephalin; both from ox brain; (c) medicinal cod liver oil; and (d) tricaprylin. The incidence of local tumors was: lecithin group, 2 of an effective total of 22 (9%); cephalin group, 2 of an effective total of 19 (10.5%); cod liver oil group, 7 of an effective total of 19 (37%); tricaprylin group, 6 of an effective total of 18 (33%). The latent period in the cod liver oil group was approximately 4 months, compared with approximately 6 months for most of the other tumors.

The rate of elimination of benzpyrene was measured and found to differ in the 4 groups, decreasing in the following order: cephalin, lecithin, cod liver oil, tricaprylin.—Authors' summary.

Diethyl Stilbestrol Excretion in Tumor-Bearing Rabbits. Bass, A. D., and Salter, W. T. [Yale Univ. Sch. of Med., New Haven, Conn.] *Yale J. Biol. & Med.*, **15**:729-733. 1943.

No significant difference was found in the excretion of diethylstilbestrol in the urine of normal rabbits and in that of rabbits with malignant tissues (adenocarcinoma of rabbit uterus) growing in the anterior chamber of the eye. The observation that diethylstilbestrol is eliminated, largely in the free state, confirms the work of previous investigators. The authors note the relatively low toxicity in the rabbit as compared with the dog. Water diuresis did not significantly alter the daily excretion of diethylstilbestrol when given orally. Colorimetric methods gave recoveries of diethylstilbestrol from rabbit urine comparable to those previously disclosed by bioassay.—J. L. M.

The Reaction of the Pituitary Gland and Related Hypothalamic Centres in the Hamster to Prolonged Treatment with Œstrogens. Vazquez-Lopez, E. [Labs. of the Imperial Cancer Research Fund, London, England] *J. Path. & Bact.*, **56**:1-13. 1944.

Prolonged treatment of the hamster with estradiol benzoate and diethylstilbestrol induced proliferation of cells of the intermediate lobe of the pituitary which invaded the pars nervosa, and in some cases extended as far as the wall of the third ventricle of the brain. Only at a late stage was the anterior lobe invaded by intermedial cells. The ganglion cells of the supraoptic nucleus became vacuolated and decreased in numbers, and finally disappeared entirely when the whole of the neurohypophysis had been invaded by intermedial cells. Only isolated cells of the paraventricular nucleus became vacuolated, but occasionally there were widely dispersed globular masses of a colloidal substance.

Two of the treated animals developed carcinoma of the head of the epididymis.

In discussing the significance of these pituitary changes in response to estrogens, the author states that the proliferative process "is best described as an adenoma-like hyperplasia of the pars intermedia analogous to similar growths of the anterior lobe in rats and mice."—R. J. L.

Effect of Diethylstilbestrol on Mammary Tumor Formation in Strain C3H Mice Fed a Low Cystine Diet. White, F. R., and White, J. [National Cancer Inst., Bethesda, Md.] *J. Nat. Cancer Inst.*, **4**:413-415. 1944.

This is an investigation of factors responsible for the complete suppression of mammary gland tumors by a low cystine diet in strain C3H mice normally showing a mammary tumor incidence of 97.43%. The results obtained indicate that the absence of tumors may be related to the lack of estrus since diethylstilbestrol pellets implanted subcutaneously raised the incidence of tumor formation from zero to 44.7%.—K. R. P.

Studies in Cancer. VII. Enzyme Deficiency in Human and Experimental Cancer. Roskelley, R. C., Mayer, N., Horwitt, B. N., and Salter, W. T. [Yale Univ. Sch. of Med., and New Haven Hosp., New Haven, Conn.] *J. Clin. Investigation*, **22**:743-751. 1943.

A series of human and animal neoplasms were investigated for their biochemical activity in relation to their pathological status. The chemical studies included cytochrome oxidase system or succinoxidase activities and aerobic glycolysis. In general, most neoplasms are deficient in the first two of these, but only frank cancers exhibit characteristic changes involving all three criteria. In animals developing neoplasia under the influence of a chemical carcinogen or of a virus, loss of cytochrome system activity definitely precedes the histological appearance of frank malignancy and the appearance of aerobic glycolysis. Neoplastic tissue that is obviously not malignant may show striking diminution in this capacity for oxidative activity. These findings are discussed (1) as chemical criteria of neoplasia and (2) as evidence that the process of carcinogenesis involves a preliminary loss of oxidase activity.—J. L. M.

Microfilm copies of such papers here abstracted as are available may be obtained from Medicofilm Service of the Army Medical Library at 25¢ for each complete article, not exceeding 25 pages in length—and 10¢ for each additional 10 pages or fraction thereof. Prepayment is not requested. Remittance may be made with subsequent orders and in such manner as found most convenient. Address—Medicofilm Service, Army Medical Library, Washington D. C

Studies in Cancer. X. Oxidative Capacity of Tumors. MAYER, N. [Yale Univ. Sch. of Med., New Haven, Conn.] *Cancer Research,* **4**:345-348. 1944.

The oxidative behavior of the homogenates of the following tumors was studied in the presence of optimal concentration of paraphenylenediamine and succinate: hepatoma induced by *p*-dimethylaminoazobenzene, rhabdomyosarcoma, lymphosarcoma, Yale tumor No. 1, and V_2 carcinoma. For comparison, similar experiments were conducted with homogenates of normal tissues, *i.e.*, rat and mouse leg muscle and normal rat liver. It was found that the tumor homogenates showed little or no increase in oxygen consumption with either paraphenylenediamine or succinate. On the contrary, homogenates of the normal homologous tissues showed a definite increase with both substrates. These observations are in accord with similar findings previously reported for surviving slices of the respective tissues named. Therefore, the response to the substrates mentioned above reflects the over-all activity of the respective oxidative systems involved.

In addition, the oxidative capacity of V_2 carcinoma slices was studied in similar fashion. They, too, exhibited only a slight increase in oxygen consumption in the presence of these substrates.—Author's abstract.

Studies on Tumors of the Testis. I. Water and Electrolyte Content of Testicular Tumors and of Normal, Cryptorchid, and Estrogenized Testis. HUGGINS, C., and EICHELBERGER, L. [Univ. of Chicago, Chicago, Ill.] *Cancer Research,* **4**:447-452. 1944.

Water, fat, chloride, sodium, potassium, calcium, magnesium, and nitrogen were determined on normal, cryptorchid, and estrogenized testes of dogs as well as on canine testicular tumors. Typical patterns of the content of water, fat, and electrolytes are described for normal testis and various physiological types of atrophy of the germinal epithelium. While definite and categorical changes occur in neutral fat, water, solids, and nitrogen, quantitative generalizations concerning electrolytes cannot be made for the testicular cancer of dogs because of the wide deviations.—Authors' abstract.

The Metabolism of Pyruvate by Normal and Leukemic White Cells. ABELS, J. C., JONES, F. L., CRAVER, L. F., and RHOADS, C. P. [Memorial Hosp., New York, N. Y.] *Cancer Research,* **4**:149-152. 1944.

From 65 to 101% of the thiamine present both in normal and leukemic white cells exists in the form of cocarboxylase. Nevertheless, those enzyme systems that are known to utilize cocarboxylase as a coenzyme could not be demonstrated in either the normal or leukemic cells. Qualitative and quantitative differences have been observed for the utilization of pyruvate by normal and leukemic white cells. The normal cells apparently utilize less pyruvate and, in most instances, convert a greater proportion of that compound to lactate than do the leukemic cells. This abnormality of the neoplastic cells probably is not due simply to their apparent youth.—Authors' abstract.

Observations on the Inhibition of Development of Spontaneous Leukemia in Mice by Underfeeding. SAXTON, J. A., JR., BOON, M. C., and FURTH, J. [Cornell Univ. Med. Coll., New York, N. Y.] *Cancer Research,* **4**:401-409. 1944.

The incidence of spontaneous lymphoid leukemia in a high-leukemia strain of mice was 65.0% in 100 normally fed control mice. In 79 related mice that received limited amounts of an otherwise adequate diet, the incidence of this disease was 10.1%. The length of life of these mice was considerably prolonged by underfeeding. Bioassays at 9 and 12 months indicated the absence of malignant lymphocytes in the underfed mice and disclosed their presence at 14, 16, and 17 months. In normally fed mice of the same strain malignant cells appeared at 7 to 13 months. These experiments indicate that (a) malignant lymphoid cells are absent in young normal mice of the high leukemia stock Ak, (b) the malignant transformation takes place shortly before leukemia becomes manifest, and (c) this transformation is delayed but not entirely prevented by underfeeding.—Authors' abstract.

Bestimmung des Doppelstickstoffes bei der polarographischen "Krebsreaktion" von Brdićka. [Determination of Double-Nitrogen in the Polarographic "Cancer Test" of Brdićka.] RICHTER, A. F. [Staedt. Ambulatorium der Hauptstadt Prag] *Naturwiss.,* **31**: 466-467. 1943.

As "double-nitrogen" the author designates the difference between peptide-rich and peptide-free filtrates prepared with the aid of certain deproteinizing reagents from sera of normal persons and of tumor patients. The serum of tumor patients gave values higher than did normal serum or serum of nephrosclerotic patients. The investigation is still in progress.—K. G. S.

Blood Proteose and Cancer. WINZLER, R. J., and BURK, D. [National Cancer Inst., Bethesda, Md.] *J. Nat. Cancer Inst.,* **4**:417-428. 1944.

The work being reported extends to experimental animals of various species the findings of Brdićka and earlier workers that sera of human cancer patients contain abnormally large amounts of protein split-products that possess the properties of proteoses.

Rat blood was found to contain a nondialyzable, heat-stable, cystine-containing proteose, non-precipitable by sulfosalicylic acid or 20 to 30% trichloracetic acid. Proteose nitrogen of sulfosalicylic acid filtrates was 3 to 4 mgm. per 100 cc. of blood in normal, and 20 to 50 mgm. in tumor-bearing rats. The dialyzable nonprotein nitrogen blood levels were essentially the same in both groups of animals.

Other conditions that led to increased blood proteose in the rat were pyogenic infections (pneumonia, cecitis) and, to a lesser extent, severe injuries and advanced pregnancy. Without notable effect were strain, sex, age, diet, liver cirrhosis, and regenerative growth of liver. Regression, or extirpation of tumors was accompanied by rapid return to normal proteose levels. In rabbits, transplanted malignant tumors (V_2 and Brown-Pearce carcinomas) gave blood proteose values several times normal, but young, homologous benign tumors (Shope papillomas) did not.

From a discussion of the results it appears probable that the excess of proteoses in the blood of tumor-bearing animals derives largely, if not wholly, from blood protein (albumin) rather than from the tumor nitrogen itself.—A. C.

Development by Ducks of Natural Neutralizing Antibodies for a Duck Variant of the Rous Sarcoma Virus. KING, J. W., and DURAN-REYNALS, F. [Yale Univ. Sch. of Med., New Haven, Conn.] *Yale J. Biol. & Med.*, **16**:53-57. 1943.

In Pekin ducks neither the egg substance nor the serum of the embryo before hatching had any neutralizing power for the Rous sarcoma virus. Such power began to appear 5 hours after hatching, was more pronounced 24 hours after hatching, and was well-developed at 3 weeks. Possibly there was a further increase in the neutralizing power with further growth of the individual. Not only did sera from embryos and from hatching ducks fail to neutralize the virus, but the tumors induced by the mixtures of such sera and virus were much larger than those induced by the control mixtures. The authors believe this result is due to the protection of the virus against spontaneous inactivation by serum colloids.

Sera stored at 2 to 4° C. for several weeks retain their neutralizing power. The neutralizing power of the serum is not suppressed by heating at 50° for 30 minutes but is suppressed by heating at 55 to 65° for 30 minutes.

Most sera diluted 1:50 and a few diluted 1:100 retain the neutralizing capacity. Sera diluted 1:5 and 1:10 are practically as active as undiluted sera.—J. L. M.

Suppression of Growth of the Brown-Pearce Tumor by a Specific Antibody. KIDD, J. G. [Rockefeller Inst. for Med. Research, New York, N. Y.] *Science*, **99**:348-350. 1944.

Earlier work by the author showed that certain rabbits bearing the Brown-Pearce carcinoma develop in the blood a specific antibody that reacts *in vitro* with a distinctive sedimentable constituent of the carcinoma cells. In the present work suspensions of living Brown-Pearce tumor cells were incubated at 37° C. for 2 to 3 hours in the presence of serum known to contain the specific antibody in high titer. At the end of the incubation the cells appeared to be unaltered, but when injected into rabbits they either failed to produce tumors, or at most gave rise to small nodules. Control injections of Brown-Pearce carcinoma cells incubated with serum from normal animals or from animals bearing other types of tumors regularly gave rise to large tumors.

In a second group of experiments cell-free extracts of Brown-Pearce carcinoma were injected intraperitoneally into normal rabbits. Those animals that developed detectable amounts of the specific antibody were found to be refractory to small doses of Brown-Pearce tumor cells, but not to implantations of other tumors. Contrariwise, normal uninjected animals, and injected animals that failed to develop the antibody were susceptible to implantations of Brown-Pearce carcinoma.

Additional studies on the growth of the carcinoma in rabbits showed that abrupt regressions occurring 3 to 4 weeks after implantation were associated with the development of high titers of antibody, while animals in which the tumor grew progressively and metastasized developed little or no antibody.—R. B.

The Prophylactic Effect of a Colloid Material on the Skins of Mice Painted with Various Types of Carcinogenic Agents. TWORT, J. M., and LYTH, R. [Christie Hosp., and Univ. of Manchester, England] *J. Hyg.*, **43**:252-255. 1944.

It has been stated that the colloid material (S.D.2.), which contains as active constituents 30% tetrachlorethylenum and 10% oleum pini, has benefited considerably certain forms of dermatitis, including that produced by mineral oil. The authors were therefore interested to discover whether it would resemble their lanolin-olive oil ointment in its effect on tumor production in mice. It was found that both colloid and lanolin retarded tumor production by benzpyrene, tar, and shale oil. Colloid was more effective than lanolin against benzpyrene and tar, but lanoline was more effective against shale oil. Colloid provided some protection if applied during the treatment of mice with benzpyrene but no protection, in fact it facilitated tumor production, if applied after cessation of the treatment.—I. H.

The Clinical Effects of Aldehyde Bisulfites in Patients with Cancer. I. The Administration of Heptylaldehyde Bisulfite to Patients with Inoperable Mammary Carcinoma Metastatic to Bone. ABELS, J. C., TREVES, N., HERRMANN, J., SINGHER, H. O., KENSLER, C. J., and RHOADS, C. P. [Memorial Hosp., New York, N. Y.] *Cancer Research*, **4**:438-443. 1944.

The daily oral administration of from 1 to 12 gm. of heptylaldehyde bisulfite for from 20 to 231 days to 11 patients with mammary carcinoma that had metastasized to the bones, did not alter significantly the expected course of the disease. Likewise the continuous intravenous administration of the compound in amounts close to toxic levels failed to influence the course of the disorder in any of 3 instances.—Authors' abstract.

The Clinical Effects of Aldehyde Bisulfites in Patients with Cancer. II. The Administration of Heptylaldehyde Bisulfite to Patients with Lymphomas. SINGHER, H. O., ABELS, J. C., CRAVER, L. F., and RHOADS, C. P. [Memorial Hosp., New York, N. Y.] *Cancer Research*, **4**:444-446. 1944.

The intravenous administration of heptylaldehyde bisulfite to 3 patients with neoplastic lymphomatous disorders had no significant effect on the clinical course of the disease in the patients studied or on the microscopic appearance or respiratory activity of involved lymph nodes.—Authors' summary.

Ultracentrifugal Studies of Some Complexes Obtained from Mouse Milk, Mammary Tumor, and Other Tissues. KAHLER, H., and BRYAN, W. R. [National Cancer Inst., Bethesda, Md.] *J. Nat. Cancer Inst.*, **4**:37-45. 1943.

Particulate components of mouse milk, C3H mammary tumor, normal chick embryo, human mammary tumors, cow's milk, and Jensen rat sarcoma were separated out by methods of differential centrifugation and examined relative to sedimentation rates and ultraviolet absorption. The two main particulate components of C3H tumor showed sedimentation rates of 62 *s* and 90 to 92 *s* and of these the faster is apparently the analogue of the

principal component in water extractions of normal chick embryo. From transplanted mammary tumors, mouse milk, and human mammary carcinoma somewhat similar components were obtained. From cow's milk and Jensen rat sarcoma, however, the particulate components were different. A molecular weight of 3 to 4 million was estimated for the macromolecular particles with a sedimentation rate of 62 *s*, and 5 million for the faster moving components. It is indicated that the particles are asymmetrical. Ultraviolet absorption studies demonstrated a high percentage of nucleic acid in the fraction from mammary tumor and pressed milk, a lower percentage in machine-obtained milk and Jensen rat sarcoma, and none in cow's milk. This information plus a positive Bial's test for pentose indicated the presence of a ribose-nucleic acid complex in the material from C3H mammary tumors.—K. R. P.

Chromosomal Changes in Epidermal Carcinogenesis. Biesele, J. J., and Cowdry, E. V. [International Cancer Research Foundation and Barnard Free Skin and Cancer Hosp., and Anatomical Lab., Washington Univ., St. Louis, Mo.] *J. Nat. Cancer Inst.*, **4**:373-384. 1944.

The authors report on a careful study of chromosomes in mouse epidermis that had been treated with methylcholanthrene in benzene. No evidence of chromosome enlargement was found in untreated or benzene-treated epidermis. In epidermis subjected to carcinogens, however, the changes were pronounced. From the second day on through carcinogenesis, diplochromosomes and even more greatly enlarged chromosomes were found. Their frequency remained at about 8% through the first 2 months of painting, but increased later in the induced carcinomas to more than 50%. Changes in frequency of enlarged chromosomes and changes in chemical composition of epidermis measured in terms of iron, calcium, magnesium, potassium, and ascorbic acid show a rough correspondence.—K. R. P.

Measurement of Epithelial Growth in Surgical Wounds of the Rabbit's Ear. Henshaw, P. S., and Meyer, H. L. [National Cancer Inst., Bethesda, Md.] *J. Nat. Cancer Inst.*, **4**:351-358. 1944.

Epithelial wounds of various shapes were made on rabbit's ears for a comparative study of methods of measuring rate and behavior of epithelial regeneration. The repair process was found to display 3 phases: (1) an initial lag phase of 3 to 5 days during which little or no coverage was displayed; (2) an active-growth phase, the extent of which was determined by the wound size; and (3) a reduced-growth phase, which terminated the healing period. The rate of lateral extension of the epithelial sheets was found to be essentially constant during the active-growth phase, and the time required for complete coverage was determined to be proportional to the breadth of the wound as measured by the diameter of the largest inscribed circle.—K. R. P.

Incidence of Spontaneous Fibroadenoma in the Albany Strain of Rats. Burack, E., Danzi, M. V., Wolfe, J. M., and Wright, A. W. [Albany Med. Coll., Union Univ., Albany, N. Y.] *Cancer Research*, **4**:410-416. 1944.

The incidence of spontaneous benign mammary fibroadenomas in the Albany strain of rats and in one subline (Line 147) was calculated by two methods: (a) by determining the percentage of tumor-bearers among animals that live to the beginning of various age groups, and (b) by computing corrected tumor rates per 10,000, using a standard population.

The tumor rates were computed for 4 successive years. It was shown that on the basis of rates per 10,000 of a standard population, the rate for Line 147 was 322 in 1943, as against 152 for the entire colony in 1940. Thus, the tumors are appearing more than twice as frequently as a result of close inbreeding. On the basis of the percentage of tumor-bearing animals among those that live to various ages, it was calculated that 42.8% of the females of Line 147 that reach the age of 9 months are destined eventually to develop tumors. For the colony as a whole, 28.7% of the animals that reach the age of 9 months will eventually have tumors. The frequency of tumor development appeared to be, in general, higher for the females that had never been pregnant than for the breeding females.—Authors' abstract.

Spontaneous Tumors of the Adrenal Cortex in a Castrated Male Rat. Heiman, J. [Columbia Univ., New York, N. Y.] *Cancer Research*, **4**:430-432. 1944.

Adrenal cortical adenomas appeared spontaneously in an old castrated male rat that was resistant to the growth of transplanted benign subcutaneous tumors. This rat was 18 months old when castrated and weighed 445 gm. at death, while other castrates of the same series, growing subcutaneously implanted benign tumors, averaged 295 gm. and showed normal but involuted adrenals. The adrenal tumors, showing an adenomatous structure were in the juxtamedullary zone; active mitoses were present.—Author's summary.

Embryonal Nephroma in a Calf. Walker, R. [Rensselaer Polytechnic Inst., Troy, N. Y.] *J. Am. Vet. M. A.*, **102**:7-10. 1943.

The growth resembled the human Wilms' kidney tumor in histologic details, but its location was entirely extrarenal. A complete autopsy was not performed. An extensive review of the occurrence of this tumor in other species is included, and there are numerous photomicrographs.—E. E. S.

Neoplasms of the Bovine Eye. Frank, E. R. [Kansas State College, Manhattan, Kans.] *J. Am. Vet. M. A.*, **102**: 200-203. 1943.

Squamous carcinoma involving one eye or its appendages is the most common neoplasm of cattle. Occasionally lymphocytoma develops in the posterior part of the orbital cavity causing exophthalmos. The paper is chiefly concerned with details of surgical eradication of the tumor.—E. E. S.

Carcinoma of the Stomach of a Dog. Davis, C. L., and Naylor, J. R. [U. S. Bureau of Animal Industry, Denver, Colo.] *J. Am. Vet. M. A.*, **102**:286-288. 1943.

Malignant epithelial tumors are common in the dog, but they usually arise in the skin or mammary region. Few gastric cancers have been reported. The one recorded here occurred in an 8 year old male chow as an ulcerated mass involving the pyloric portion of the stomach.

The growth was highly undifferentiated and had invaded the lymphatics, but no metastases were found.—E. E. S.

Adenocarcinoma of the Kidney in a Dog. HAIGLER, S. W. [St. Louis, Mo.] *J. Am. Vet. M. A.,* **102**: 469-471. 1943.

The tumor, a well-differentiated growth replacing the greater part of the kidney, was removed surgically.—E. E. S.

Experimental Gastric Carcinoma. NETTLESHIP, A. [National Cancer Inst., Bethesda, Md.] *Arch. Surg.,* **46**:793-806. 1943.

This review includes a discussion of the experimental approaches made in the study of cancer of the stomach, the possible relationship of abnormal conditions of gastric mucosa that may be associated with carcinoma, environmental and intrinsic factors that affect the mucosa, and suggestions of lines for further experimentation.—W. A. B.

Clinical and Pathological Reports

The Racial Distribution of Cancer. I. Epithelial Tumors of the Skin, Lip, and Breast. SCHREK, R. [Edward Hines, Jr., Memorial Hosp., Hines, Ill.] *Cancer Research,* **4**:433-437. 1944.

The method consisted in determining what percentage of patients with a given tumor were colored. This percentage colored was compared with that obtained for a control group which comprised all patients with cancer except those with certain types of tumor (cancer of the skin, lip, uterus, and cervix). The data analyzed by this means were from records of Edward Hines, Jr., Memorial Hospital, the U. S. Public Health survey on the prevalence of cancer, and the Mortality Statistics.

Carcinoma of the exposed skin and of the lip, and keratosis of the skin, had very low percentages colored. In contrast, carcinoma of the covered skin had approximately the same percentage colored as the control group.

Cancer of the breast in the male had a relatively high percentage colored. The percentages colored for tumor of the breast in the female was slightly elevated. Observed differences in the percentage colored for a particular tumor and for the control group were studied to determine whether they were due to fortuitous factors, inherent intracellular or extracellular racial factors, or external environmental factors.—Author's abstract.

Skin and Subcutaneous Tissues

Cancer Associated with Acanthosis Nigricans. Review of Literature and Report of a Case of Acanthosis Nigricans with Cancer of the Breast. CURTH, H. O. [Coll. of Physicians and Surgeons, Columbia Univ., New York, N. Y.] *Arch. Surg.,* **47**:517-552. 1943.

To the 395 reported cases of acanthosis nigricans, a case associated with cancer of the breast is added, and the literature is reviewed. Approximately 50% of all cases of acanthosis nigricans are associated with cancer. This "malignant" type occurs mostly in middle-aged patients, and the fatal outcome is due to the accompanying tumor. In the "benign" or "juvenile" form of acanthosis nigricans the lesion, identical histologically with that in the "malignant" type, is present usually at birth or in youth and is of indefinite duration. Cancer associated with acanthosis nigricans occurs with equal frequency in the two sexes. The author found abdominal cancer in 93.67% of cases, with gastric cancer providing 69.86%. The cancer has also been found primary in the lungs, liver, breast, uterus, ovary, and other organs.

The possible etiologic relation between acanthosis nigricans and cancer is discussed. An extensive bibliography is appended.—W. A. B.

A Recurrent Tumour of Mesenchyme in an Adult. GILMOUR, J. R. [St. Andrew's E.M.S. Hosp., Billericay, Essex, England] *J. Path. & Bact.,* **55**:495-498. 1943.

A benign but recurrent tumor of the subcutis of the back in an adult woman was composed of mucous connective tissue containing neoplastic capillaries, lipoblasts, primitive erythroblasts and erythrocytes, and lymphocytoid wandering cells. It differed from mesenchyme in that focal excess of mucus had led to the formation of cystic spaces which had become lined with mesothelium. The designation "mesenchymoma" is suggested.—A. H.

Extensive Destructive Ulcerating Angioma, Nine Years After Treatment. KAPLAN, I. I. [Bellevue Hosp., New York, N. Y.] *Urol. & Cutan. Rev.,* **47**:320-321. 1943.

An extensive angioma of the face, present at birth and treated when the child was 9 months old, responded unusually well to repeated small doses of radium.—V. F. M.

Nervous System

Meningioma at the Internal Auditory Meatus. COHEN, I. [Mt. Sinai Hosp., New York, N. Y.] *J. Mt. Sinai Hosp.,* **10**:206-207. 1943.

The relatively infrequent occurrence of meningioma in the posterior fossa as compared to its occurrence above the tentorium is apparent from the published statistics. Further subdivision of these tumors in the posterior fossa has been made by splitting off a small group characterized by a symptomatology resembling that of the acoustic tumors. The author reports a case of a small meningioma in the cerebellopontine angle, not only because of its comparative rarity, but because of the abrupt onset of the eighth nerve symptoms, the irritative phenomena of the seventh nerve, and the operative trauma to the *nervus intermedius.*—A. Cnl.

Posterior Displacement of the Calcified Pineal in Subtentorial Brain Tumors. HAWES, L. E., and MEAD, S. [Massachusetts General Hosp., Boston, Mass.] *Radiology,* **40**: 367-370. 1943.

Three cases of subtentorial brain tumor are reported in which the lateral roentgenogram of the skull showed the calcified pineal body to be displaced posteriorly. This apparently paradoxical shift is thought to be explained by internal hydrocephalus caused by obstruction at the aqueduct.—R. E. S.

The Diagnosis and Prognosis of Brain Tumors. HORRAX, G. [Lahey Clinic, Boston, Mass.] *Surg. Clin. North Am.,* **23**:793-802. 1943.

After discussing brain tumors from the standpoint of diagnosis, situation, pathology, and prognosis, the author

reviews his own series of about 400 verified brain tumors observed from 1932 to 1939. More than half the tumors (218) were favorable for extirpation. They included meningiomas, acoustic neuromas, pituitary adenomas, gliomatous and hemangiomatous cysts of the cerebellum, and a rather large group of uncommon tumors. Sixty-eight per cent of the 218 patients are living and actively engaged in some form of work. Based on the number of patients who survived operation, the figure of useful life rises to 78%.—J. L. M.

Dermoid and Epidermoid Tumors (Cholesteatomas) of the Central Nervous System. Report of Twenty-Three Cases. Rand, C. W., and Reeves, D. L. [Los Angeles, Calif.] *Arch. Surg.*, **46**:350-376. 1943.

A review of the literature and report of 23 cases, 11 of which are instances of diploic or cranial epidermoid.—W. A. B.

Eye

Malignant Melanomas of the Uvea and Their Prognosis. Burch, F. B. [St. Paul, Minn.] *Minnesota Med.*, **26**:208-210. 1943.

The author's experience with 113 cases of malignant melanoma of the uvea led to the following conclusions: The result of even intensive irradiation is uncertain in all types of the disease. Roentgen therapy offers no assurance against metastasis. It is possible that when complete figures covering a longer period are available, it will appear that metastasis develops earlier after irradiation used prophylactically than when it is not so employed. At present it is believed that irradiation should be reserved for cases in which (1) the tumor-containing eye is the only one retaining vision, (2) the patient refuses enucleation, and (3) the tumor has already invaded the orbit and adjacent structures. Early diagnosis and immediate enucleation remain of the greatest value in prognosis.—J. L. M.

Prognosis of Malignant Melanomata of the Uvea from the Standpoint of Pathogenesis, Cytology and Fiber Content. Camp, W. E. [Minneapolis, Minn.] *Minnesota Med.*, **26**: 210-212. 1943.

Follow-up studies of 90 patients with malignant melanoma of the uvea were completed after a 6 year period. The tumors were grouped for study according to the classification proposed by Callendar. As an aid to prognosis a modification of Foot's stain was used to determine the "fiber content" in specimens of tissue from 29 patients who were followed for a period of 6 years or more. Results are tabulated and discussed.—J. L. M.

Pigment Freckles of the Iris (Benign Melanomas): Their Significance in Relation to Malignant Melanoma of the Uvea. Reese, A. B. [Inst. of Ophth., New York, N. Y.] *Am. J. Ophth.*, **27**:217-226. 1944.

A malignant melanoma of the uvea may be accompanied by multiple benign melanomas. Such instances represent a multiple origin of the tumor, and the iris is a common site for the benign feature.—E. C. R.

Epithelioma of the Conjunctiva. Titche, L. L. [Vet. Admin., Oteen, N. C.] *M. Bull. Vet. Admin.*, **20**:449-450. 1944.

Case reported because of the infrequency of this condition.—M. E. H.

Breast

Regression of Tumours Following Treatment by Stilbœstrol and X-Ray Therapy, with Notes on a Case of Breast Tumour Which Regressed with Stilbœstrol Alone. Binnie, G. G. [North Staffordshire Royal Infirmary, Stoke-on-Trent, England] *Brit. J. Radiol.*, **17**: 42-45. 1944.

A record of the present state of four cases treated recently, illustrated by radiographs and photographs. For details of treatment the original paper must be consulted.

Case 1.—Recurrence after amputation of breast for carcinoma; no microscopic examination; metastases in sternum and lung, with pleural effusion; patient very dyspneic. Deep x-ray therapy and stilbestrol (6 mgm. later reduced to 2 mgm. daily). Disappearance of metastasis in lung and almost complete absorption of the effusion.

Case 2.—Amputation of breast for Paget's disease; 3 months later, nodule on chest wall treated by radium needles. Secondary deposits in lungs, pleural effusion. X-ray therapy and stilbestrol (3 mgm. daily, increased to 6 mgm.); some lung metastases disappeared.

Case 3.—Ulcerating mass of spheroidal-celled carcinoma in axillary tail of breast; deep x-ray therapy and stilbestrol (2 mgm. daily, increased to 7 mgm.). Tumor reduced in size, and surface healed.

Case 4.—Patient aged 76 years. Swelling in breast of 5 years duration, now fungating mass. Stilbestrol only, (3 mgm. to 7 mgm.). Shrinkage of tumor, later "healing was complete." No microscopic examination.

The nature of the tumor in Case 1 is not proved, but if it is malignant "response is such as could not have been expected from the radiation treatment alone." In Case 2, regression of large metastasis in the lung began only after raising the dose of stilbestrol from 3 mgm. to 6 mgm. daily. In Case 3 the patient improved with moderate doses of x-rays alone, but "more pronounced improvement on smaller doses of X rays when given with stilbestrol is not what I should have expected if stilbestrol had no action."—E. L. K.

Carcinoma of the Breast in the Male. Report of Case. Niethammer, W. A., and Dublin, W. B. [Tacoma, and Fort Steilacoom, Wash.] *Northwest Med.*, **42**:368-369. 1943.

An ulcerating adenocarcinoma occurred in the left breast of a white male aged 55 years. Skin nodules were found in the vicinity, and the axillary lymph nodes contained tumor. The patient died 7 months after radical mastectomy and was found to have widespread metastases as well as local recurrence of the tumor.—E. E. S.

Female Genital Tract

The Krukenberg Tumor: A Critique, with the Report of an Additional Four Cases, Including the Smallest on Record. Copland, S. M., and Colvin, S. H. [Touro Infirmary, New Orleans, La.] *Am. J. Obst. & Gynec.*, **45**:59-69. 1943.

A report of 4 cases, with discussion.—A. K.

The Management of Ovarian Tumors in Elderly Women. Goldsmith, J. W. [Kings County Hosp., Brooklyn, N. Y.] *Am. J. Obst. & Gynec.*, **45**:518-521. 1943.

Three hundred and forty-four cases of ovarian tumor are summarized. Of 88 neoplasms found in women of 50 or more years of age, 30.7% were benign, and with proper precautions they were successfully removed. The routine for the care of this group of patients is outlined.—A. K.

Malignant Dysgerminoma of the Ovary. A Fatal Case with Autopsy. Kirschbaum, J. D., and Newman, B. [Woodlawn Hosp.; South Shore Hosp.; and Northwestern Univ., Chicago, Ill.] *Am. J. Obst. & Gynec.*, **45**:337-340. 1943.

A case of dysgerminoma of the right ovary in a patient 36 years of age is described. Three years subsequent to surgical removal, and following x-ray therapy, the patient died. At necropsy there were extensive metastases to the peritoneum, lungs, both kidneys, and the liver.—A. K.

Special Ovarian Tumors: With a Report of a Series of Cases. Long, C. H., and Ziskind, J. [Johnson City, Tenn., and Tulane Univ., New Orleans, La.] *J. Tenn. M. A.*, **36**:5-12. 1943.

In a series of 98 solid ovarian tumors discovered at operation at Charity Hospital in New Orleans, 25 belonged to the group of ovarian neoplasms that display biologic activity. Ten were classified as granulosa cell tumors; 4 as theca cell tumors; 1 as an arrhenoblastoma; 1 as an adrenal cell carcinoma; 2 as dysgerminomas; and 7 as Brenner tumors. The pathologic and clinical characteristics of each type are presented in detail.—E. E. S.

Malignant Lymphangioma of the Ovary. Rice, M., Pearson, B., and Treadwell, W. B. [Louisiana State Univ. Sch. of Med. and Charity Hosp., New Orleans, La.] *Am. J. Obst. & Gynec.*, **45**:884-891. 1943.

This is the fifth case of lymphangioma of the ovary to be reported. Morphologically the tumor was benign at the time a resection was done. Six months later local recurrence, peritoneal dissemination, hepatic metastasis, and a new growth in the other ovary appeared. The tumor was then morphologically malignant. Because of the definite necrosis of the primary tumor together with the pronounced hepatic necrosis present at autopsy, the authors felt that the tumor was malignant at its inception in spite of its benign morphological appearance.—A. K.

Brenner Tumor of the Ovary Complicating Pregnancy. Rogers, F. S. [George Washington Univ. Sch. of Med., Washington, D. C.] *Am. J. Obst. & Gynec.*, **45**:896-898. 1943.

This is the fifth case reported in which a Brenner tumor has been associated with pregnancy. The tumor was removed without disturbance of the normal course of the pregnancy.—A. K.

Sarcoma of the Uterus. A Clinicopathologic Study of Twenty-Seven Cases. Bosse, M. D., and Stanton, J. N. [Western Pennsylvania Hosp., Pittsburgh, Pa.] *Am. J. Obst. & Gynec.*, **45**:262-268. 1943.

In sarcoma of the uterus gross and microscopic evidence of invasiveness are the important criteria of malignancy; such microscopic features as increase in cellularity, variability in cellular morphology, anaplasia, giant cells, hyperchromatic nuclei, and even mitoses are less reliable criteria and even may be quite misleading.—A. K.

Hydradenoma Vulvae. Danforth, W. C. [Evanston, Ill.] *Am. J. Obst. & Gynec.*, **45**:329-333. 1943.

A report of 2 cases. This type of tumor is a benign neoplasm that apparently arises from the vulvar sweat glands. Clinically these tumors are considered to be important since they may be confused with carcinoma; their true nature should be recognized lest radical surgical procedures be undertaken. Complete excision of the tumor usually represents satisfactory treatment; electrocoagulation also is used.—A. K.

Benign Papillary Epithelioma of the Vulva. Ehrlich, H. E., and Horowitz, E. A. [Beth Israel Hosp., New York, N. Y.] *Am. J. Obst. & Gynec.*, **45**:879-884. 1943.

A case of benign papillary epithelioma of the vulva is described, and 4 similar cases are collected from the literature. The tumor occurs as a single, round or ovoid mass, whose surface is papillary and nonulcerated.—A. K.

Degenerating Fibroblastic Sarcoma of the Uterus Complicating a Pregnancy. Igna, E. J., and Mitchell, W. B. [Grace Hosp., Detroit, Mich.] *Am. J. Obst. & Gynec.*, **45**:303-309. 1943.

A report of a case. A second case is given in an addendum. Sarcoma comprises only 4.5% of uterine malignant growths; sarcomatous change occurs in less than 2% of the uterine myomas; sarcomatous alteration of a myoma complicates only 0.0044% of pregnancies. Pain is the cardinal symptom in such complications. Operative removal of the sarcomatous myoma gives a better prognosis for a full-term pregnancy. Diagnosis of sarcoma is usually dependent on microscopic examination.—A. K.

Endometriosis. A Study of One Hundred and Seventeen Cases with Special Reference to Constricting Lesions of the Rectum and Sigmoid Colon. Jenkinson, E. L., and Brown, W. H. [St. Luke's Hosp., Chicago, Ill.] *J. A. M. A.*, **122**:349-354. 1943.

Constricting lesions of the rectosigmoid due to endometriosis are of sufficient frequency to warrant careful consideration before extensive resection of the colon or rectum is attempted.—M. E. H.

Bilateral Adenocarcinoma of Fallopian Tubes. Mullins, D. F., and Mosteller, R. [Emory Univ. Sch. of Med., Atlanta, Ga.] *Am. J. Obst. & Gynec.*, **45**:1042-1044. 1943.

A case report.—A. K.

Rhabdomyosarcoma with Adenocarcinoma of the Uterus. Poole, C. S. [Mount Sinai Hosp., New York, N. Y.] *Am. J. Obst. & Gynec.*, **46**:434-440. 1943.

A report of a case, with discussion.—A. K.

Iliac Lymphadenectomy for Group II Cancer of the Cervix. Technique and Five-Year Results in 175 Cases. Taussig, F. J. [Barnard Free Skin and Cancer Hosp., St. Louis, Mo.] *Am. J. Obst. & Gynec.*, **45**:733-748. 1943.

One hundred and seventy-five cases of group II cervical cancer were treated by iliac lymphadenectomy and radiation of the primary tumor. Iliac gland removal is considered a simple and safe procedure. The 5 year survival rate was 38.6% (70 cases) as compared with 22.9% (118

cases) in which treatment with radiation alone was used. Cancer was found present in the lymph nodes in 26.8% of the 175 cases operated upon. Even in those cases with demonstrated mestastases, a 5 year salvage of 21% was obtained.—A. K.

Carcinoma and Sarcoma in the Same Uterus. Watson, B. P. [Sloane Hosp. for Women, and Columbia Univ., New York, N. Y.] *Am. J. Obst. & Gynec.*, **45**:1025-1031. 1943.

A case report.—A. K.

Hydatiform Mole and Associated Tumors of the Chorion. Williams, T. J. [Univ. of Virginia, University, Va.] *Am. J. Obst. & Gynec.*, **45**:432-444. 1943.

A discussion of hydatiform mole with a review of 24 cases is presented. If the mole is benign and its passage complete, recovery may be uneventful. In one-half the cases reviewed chorionic tissues remained in the uterus and caused persistent positive biologic pregnancy tests. These cases were classified as syncytial endometritis or syncytioma (infiltration with syncytium only), malignant mole or chorioadenoma (syncytium, Langhans' cells, and villi), or chorionepithelioma (Langhans' cells and syncytium). Patients with tumors diagnosed on this basis as chorionepithelioma died, whereas all of the others survived.—A. K.

Intrathoracic Tumors—Lungs—Pleura

Primary Carcinoma of the Bronchus, with Case Reports. Gaines, J. E. [Veterans Admin., Legion, Tex.] *M. Bull. Vet. Admin.*, **20**:141-160. 1943.

Evidence seems to point to an increasing occurrence of primary carcinoma of the lung, the great majority of the growths being bronchiogenic in origin. The etiology and symptoms are discussed. Early diagnosis together with the advance in thoracic surgery made in recent years offers some hope to patients suffering from primary pulmonary carcinoma. The 5 cases reported had certain individual features of interest since all were confused with tuberculosis.—M. E. H.

Incidence of Dysphagia from Intrathoracic Extraesophageal Tumors. Hill, H. N., and Vinson, P. P. [Med. Coll. of Virginia, Richmond, Va.] *Pennsylvania M. J.*, **46**:814-815. 1942-43.

Fifty cases of mediastinal tumor have been reviewed with reference to pressure on the trachea, bronchi, and esophagus.—J. L. M.

Intrathoracic Neuroblastoma. Case Report. Lee, W. E., and Ritter, J. A. [Graduate Hosp., Univ. of Pennsylvania, Philadelphia, Pa.] *Ann. Surg.*, **117**:93-99. 1943.

A case is reported in which the diagnosis was made by aspiration biopsy and confirmed at operation.—W. J. B.

Obstructive Phenomena Associated with Primary Bronchial Cancer. Report of an Unusual Case. Ozlin, W. J., Bigger, I. A., and Vinson, P. P. [South Hill, Va. and Med. Coll. of Virginia, Richmond, Va.] *Am. J. Roentgenol.*, **50**:207-209. 1943.

A case report.—E. H. Q.

Gastrointestinal Tract

Two Cases of Œsophagectomy and Anterior Œsophagoplasty. Brock, R. C. *Proc. Roy. Soc. Med.*, **37**: 38. 1943.

Descriptions of cases.—E. L. K.

Malignant Disease at the Anus. Drueck, C. J. [Chicago, Ill.] *Urol. & Cutan. Rev.*, **47**:432-435. 1943.

This is a description of malignant neoplastic disease at the anus. A case of anal carcinoma in a male of 56, and one of anal sarcoma in a boy of 5, are described and illustrated.—V. F. M.

Carcinoma of the Stomach. A Plea for Early Diagnosis and Surgical Treatment. Hoon, M. R. [Pittsburgh, Pa.] *Pennsylvania M. J.*, **46**:929-932. 1942-43.

A brief general discussion.—J. L. M.

Carcinoma of the Duodenum. Howard, J. W. [Pondville Hosp., Wrentham, Mass., Masaschusetts Dept. Pub. Health, Walpole, Mass.] *Am. J. M. Sc.*, **206**:735-746. 1943.

Report of 3 cases, with review of the literature and discussion.—J. G. K.

The Elimination of Colostomy in the Radical Treatment of Cancer of the Large Bowel. Babcock, W. W., and Bacon, H. E. [Temple Univ., Philadelphia, Pa.] *Pennsylvania M. J.*, **46**:1143-1148. 1942-43.

A review of the subject is presented. Of 414 radical operations for cancer of the colon, the mortality rate was 6.6% for 212 cases of abdominoperineal proctosigmoidectomy without colostomy and 4% for perineal proctectomy.

Of 144 patients traced after operation, 81% were living 1 to 5 years, 38% from 5 to 10 years, and 19% ten or more years after operation.—J. L. M.

Early Diagnosis of Cancer of the Colon. Estes, W. L., Jr. [Bethlehem, Pa.] *Pennsylvania M. J.*, **46**:1139-1142. 1942-43.

The author referred a review that had been made of 40 cases of proved carcinoma of the colon. In 85% of the patients the first complaint had been abdominal pain or intermittent colic, associated with constipation, which was relieved by evacuation of gas or defecation not accompanied by any obvious change in stools. Upon recognition that these symptoms are suggestive of cancer of the large intestine, an exhaustive investigation of patients presenting them was undertaken. As a result, in the next 1½ years patients with cancer of the colon were admitted for treatment at a far earlier stage than before, and the author's resectability rate for cancer of the colon increased from 45% to 85%.—J. L. M.

Diagnosis and Surgical Aspects of Carcinoma of the Colon. Jones, T. E. [Cleveland, Ohio] *Pennsylvania M. J.*, **46**:208-212. 1942-43.

This discussion includes the following topics: carcinoma of the cecum, transverse colon, rectum and rectosigmoid, and management of the colostomy.—J. L. M.

Polypoid Lesions of the Colon of Children. Kennedy, R. L. J., Dixon, C. F., and Weber, H. M. [Mayo Clinic, Rochester, Minn.] *Surg., Gynec. & Obst.*, **77**:639-644. 1943.

Clinical discussion based on 11 cases. In 9 of the cases the growths were adenocarcinomas; in the remain-

ing 2 they were adenomas. Careful search for all such lesions should be made and their excision is advised.—J. G. K.

Carcinoma of the Transverse Colon in a Boy of Sixteen. Moes, M. J., and Dittmer, M. E. [Dubuque and Colesburg, Iowa] *J. Iowa M. Soc.*, **33**:514-516. 1943.

Report of a case. Dramatic hemorrhage, which caused hospitalization, was preceded by attacks of abdominal cramps for about 1 year. Correct x-ray diagnosis was followed by successful resection and apparent cure at the time the patient was discharged 12 weeks later.—A. C.

Carcinoma of the Right and Left Colon, Including the Rectum. Shallow, T. A., and Haskell, B. F. [Philadelphia, Pa.] *Pennsylvania M. J.*, **46**:1263-1266. 1942-43.

The authors studied the results of 100 surgically treated cases of carcinoma of the large intestine. These patients were operated upon with the definite intention of stretching the operability to its maximum degree, so that all the cases of carcinoma of the large bowel admitted to the Jefferson Hospital between 1939 and 1942 were explored. The following subjects are discussed: operability, pathology and its relationship to symptomatology, diagnosis, treatment, and type of operation.—J. L. M.

Primary Resection (Closed Anastomosis) of the Colon and Rectosigmoid. Including Description of Abdomino-Anal Methods for Restoration of Continuity of Accompanying Excision of Carcinoma of the Rectal Ampulla. Wangensteen, O. H. [Minneapolis, Minn.] *Surgery*, **14**:403-432. 1943.

A description of technics.—W. A. B.

Liver

Successful Resection of Carcinoma of the Bile Duct. Cattell, R. B. [Lahey Clinic, Boston, Mass.] *Surg. Clin. North Am.*, **23**:747-752. 1943.

Primary carcinoma of the main bile passages is infrequent. The diagnosis is usually made only at the time of exploration. A successful resection of a primary carcinoma of the common hepatic, cystic, and common bile duct is reported 7 months after operation.—J. L. M.

Hæmangiectatic Hepatoma. Heritage, K. *Proc. Roy. Soc. Med.*, **37**:40. 1943.

Description of a case.—E. L. K.

Chorionepithelioma with Regression of the Primary Uterine Tumor. Maun, M. E., and Green, W. M. [Wayne Univ. Coll. of Med., Detroit, Mich.; and St. Joseph's Mercy Hosp., Pontiac, Mich.] *Am. J. Obst. & Gynec.*, **46**:738-743. 1943.

Report of a case of chorionepithelioma of the liver. There was evidence of the previous existence of chorionepithelioma in the uterus, as judged by a hemorrhagic area. Theories of the origin of extrauterine chorionepithelioma are briefly discussed.—A. K.

Carcinoma Arising in Congenital Cysts of the Liver. Willis, R. A. [Alfred Hosp., Prahan; and Univ. of Melbourne, Victoria, Australia] *J. Path. & Bact.*, **55**:492-495. 1943.

A case is described in which carcinoma arose diffusely and progressively from the epithelium lining multiple developmental cysts of the liver (in a woman aged 27). Metastases were present in the liver itself and in the lymph nodes and lungs. Mitotic figures in the tumor cells had peculiar characters (coarse spiremes and chromosomes, and widely varying chromosome numbers).—A. H.

Bone and Bone Marrow

Notes on an Early Bone Cyst. Bennett, C. B. [Berkeley, Cal.] *Arch. Surg.*, **46**:608-610. 1943.

A case report.—W. A. B.

Case of Solitary Myeloma of the Skull. Esposito, J. J. [Coll. of Physicians and Surgeons, Columbia Univ., and the Presbyterian Hosp., New York, N. Y.] *Radiology*, **40**:195-197. 1943.

A case report.—R. E. S.

Development of Bone in Relation to the Formation of Neoplasms. Haldeman, K. O. [San Francisco, Calif.] *Radiology*, **40**:247-252. 1943.

A general discussion.—R. E. S.

Solitary Benign Enchondroma of Bone. Jaffe, H. L., and Lichtenstein, L. [Hosp. for Joint Diseases, New York, N. Y.] *Arch. Surg.*, **46**:480-493. 1943.

A discussion based on 30 cases. The lesion has a predilection for bones of the extremities; 14 were found in fingers, 6 in metacarpals, 5 in humeri, 1 in a toe, and 1 in a metatarsal. It usually involves 1 bone only in each patient. The tumor arises usually in the metaphysis and often thins and distends the cortex. The clinical symptoms ordinarily are mild and consist of swelling, pain, and tenderness. Roentgenograms aid in the diagnosis. Surgical intervention is the treatment of choice.—W. A. B.

Comments and Queries on Primary Benign and Malignant Tumors of Bone. Meyerding, H. W. [Mayo Foundation, Univ. of Minn., Rochester, Minn.] *Surg. Clin. North Am.*, **23**:1012-1029. 1943.

Concise answers are given to some of the questions concerning classification, diagnosis, and prognosis that often arise when bone tumors are discussed. Seven case reports are presented.—J. L. M.

Paget's Disease with Associated Osteogenic Sarcoma. Report of Three Cases. Pike, M. M. [Hartford, Conn.] *Arch. Surg.*, **46**:750-754. 1943.

Among 53 recorded cases of Paget's disease of bone, bone sarcoma was present in 4 (8%).—W. A. B.

An Operation for Benign Cyst of the Upper Humeral Metaphysis. Rogers, W. A. [Boston, Mass.] *Arch. Surg.*, **46**:759-761. 1943.

Two full cortical thickness grafts from the tibia are fastened in place with screws of vitallium or stainless steel after removal of the cyst lining.—W. A. B.

Adrenal

A Case of Adrenal Carcinoma and Its Hormone Diagnosis. Anderson, A. F., Hain, A. M., and Patterson, J. [Royal Infirmary and Inst. of Animal Genetics, Edinburgh, Scotland; and Horton Emergency Hosp., Epsom, England] *J. Path. & Bact.*, **55**:341-349. 1943.

A woman aged 25 years with amenorrhea and slight hirsutism was found to be excreting 15 mgm. of preg-

nanediol per day. Prolonged amenorrhea excluded the corpus luteum as the source, pregnancy was excluded both by clinical examination and by a negative Aschheim-Zondek test, and arrhenoblastoma had also been excluded. A diagnosis was made of hyperplasia or tumor of the adrenal cortex. Pregnanediol estimations were continued and showed a steady rise in daily output, while the 17-ketosteroid excretion (215 mgm. daily) substantiated the provisional diagnosis of adrenal tumor. At autopsy an adrenal carcinoma was found in place of the left suprarenal gland, and secondary deposits were discovered in the liver. The case is regarded as noteworthy on account of the mild character of the symptoms, the large size of the tumor (1780 gm.) and the rapidity of its growth, and the diagnostic aid provided by estimation of the hormone excretion.—A. H.

Bilateral Carcinoma of the Adrenal Cortex with Metastasis to Iliac Bone. Cleveland, M., and Knox, L. C. [St. Luke's Hosp., New York, N. Y.] *Arch. Surg.*, **47**: 192-202. 1943.

Report of a case.—W. A. B.

Cushing's Syndrome in Children. Review of the Literature and Report of a Case. Farber, J. E., Gustina, F. J., and Postoleff, A. V. [Edward J. Meyer Memorial Hosp., and the Univ. of Buffalo Sch. of Med., Buffalo, N. Y.] *Am. J. Dis. Child.*, **65**:593-603. 1943.

The case reported, exhibiting the typical features of Cushing's Syndrome, was due to a carcinoma of the adrenal cortex. Autopsy revealed metastases in the lungs, kidneys, and veins. Tyenty-six cases collected from the literature are reviewed.—C. J. M.

Fetal Dystocia Due to Neuroblastoma of the Adrenals with Metastases to the Liver. Weinberg, T., and Radman, H. M. [Sinai Hosp., Baltimore, Md.] *Am. J. Obst. & Gynec.*, **46**:440-444. 1943.

A case report. The probable way in which the dystocia was caused, with resulting death of the fetus, is discussed.—A. K.

Miscellaneous

Surgical Experiences with Malignant Tumors. Baker, J. W. [Virginia Mason Tumor Clinic, Seattle, Wash.] *Northwest Med.*, **42**:186-190. 1943.

Case reports of Paget's disease of the breast, carcinoma in perforated peptic ulcer, total gastrectomy for polypoid gastric carcinoma, a small renal carcinoma, and of carcinoma of the anus are very briefly presented.—E. E. S.

Eight Years' Experience in Cancer in Twenty-One Connecticut Hospitals. Macdonald, E. J. [State Dept. of Health, Hartford, Conn.] *Connecticut M. J.*, **7**:536-537. 1943.

Progress is reported on the plan to make available to the medical profession the resources of the hospital record offices so that the information on individual case histories for cancer could be massed for analysis. Twenty-one hospitals comprising 84% of total hospital beds in the State are participating in the plan.—M. E. H.

Laboratory Aids in the Diagnosis of Endocrine Tumors. Perkin, H. J. [Lahey Clinic, Boston, Mass.] *Surg. Clin. North Am.*, **23**: 845-849. 1943.

This paper outlines briefly some of the laboratory procedures employed at the Lahey Clinic that have been found of value in confirming the presence of tumors involving the following organs: pituitary body, pineal body, parathyroid, thyroid, pancreas, adrenal, ovary, prostate, and testis.—J. L. M.